Journal of
DAIRY SCIENCE

Vol. 42 October, 1959 Number 10

CONTENTS

People and Events 3

From the Secretary's Desk 20

Research Papers:

Applications and Limitations of Quality Tests for Milk and Milk Products. A Review. **C. K. Johns** 1625

Characteristics of High-Melting Triglyceride Fractions from the Fat-Globule Membrane and Butter Oil of Bovine Milk. **M. P. Thompson, J. R. Brunner, and C. M. Stine** 1651

Comparison of Nucleic Acids and Other Phosphorus Compounds of Terramycin-Sensitive and -Resistant Strains of Streptococcus Lactis. **K. M. Shahani** 1659

Use of the Selection Index Method to Account for the Number of Daughters and Records in Progeny-Testing a Dairy Bull. **S. R. Searle** . . . 1666

A Comparison of Orchardgrass-Ladino Clover and Orchardgrass as Pasture for Milking Dairy Cows. **F. R. Murdock, A. S. Hodgson, and H. M. Austenson** 1675

A Comparison of the Relative Efficiency of Three Pasture Utilization Systems. **C. H. Gordon, O. J. Hunt, G. R. Mowry, and W. R. Harvey** . . . 1686

Some Factors Affecting Weight Gains of Dairy Heifers Fed All-Roughage Rations. **M. E. McCullough and W. E. Neville, Jr.** 1698

Nutrient Losses, Quality, and Feeding Values of Wilted and Direct-Cut Orchardgrass Stored in Bunker and Tower Silos. **C. H. Gordon, E. A. Kane, J. C. Derbyshire, W. C. Jacobson, C. G. Melin, and J. R. McCalmont** 1703

Effect of Aureomycin, Erythromycin, and Hygromycin on the Growth Rate and Well-Being of Young Dairy Calves. **C. A. Lassiter, L. D. Brown, and C. W. Duncan** 1712

Unusual Magnitude and Variation of Plasma Protein-Bound Iodine Values of Dairy Cattle. **R. O. Asplund, G. A. McLaren, H. O. Henderson, and I. D. Porterfield** 1718

Technical Note:

Evaluation of Short-Interval Milking as a Physiological Technique. **D. R. Lamond and W. V. Candler** 1724

(Continued on inside front cover)

CONTENTS

(Continued from front cover)

Our Industry Today:

Dry vs. Lye-Solution Storage of Milking Machine Rubber Inflations. J. M. Jensen 1726

Recent Developments in Automatic Cleaning of Storage Tanks. V. E. Meyers 1730

Our Association:

Call for Papers for the 1960 Annual Meeting of the American Dairy Science Association. N. L. Jacobson 1734

Nominations for Vice-President and Directors of A.S.D.A. 1735

Report of Representative to the United States Livestock Sanitary Association. C. G. Bradt 1735

Some Current Aspects of Animal Disease Control. L. A. Black and W. D. Pounden 1738

Abstracts of Papers Presented at the Western Division Meeting 1740

Book Reviews 1744

AMERICAN ASSOCIATION
FOR THE ADVANCEMENT OF SCIENCE
SECTION O AGRICULTURE

SYMPOSIUM

Germ Plasm Resources in Agriculture: Development and Protection

DECEMBER 28 TO 31, 1959

Exhibit Hall (South), Morrison Hotel, Chicago, Illinois

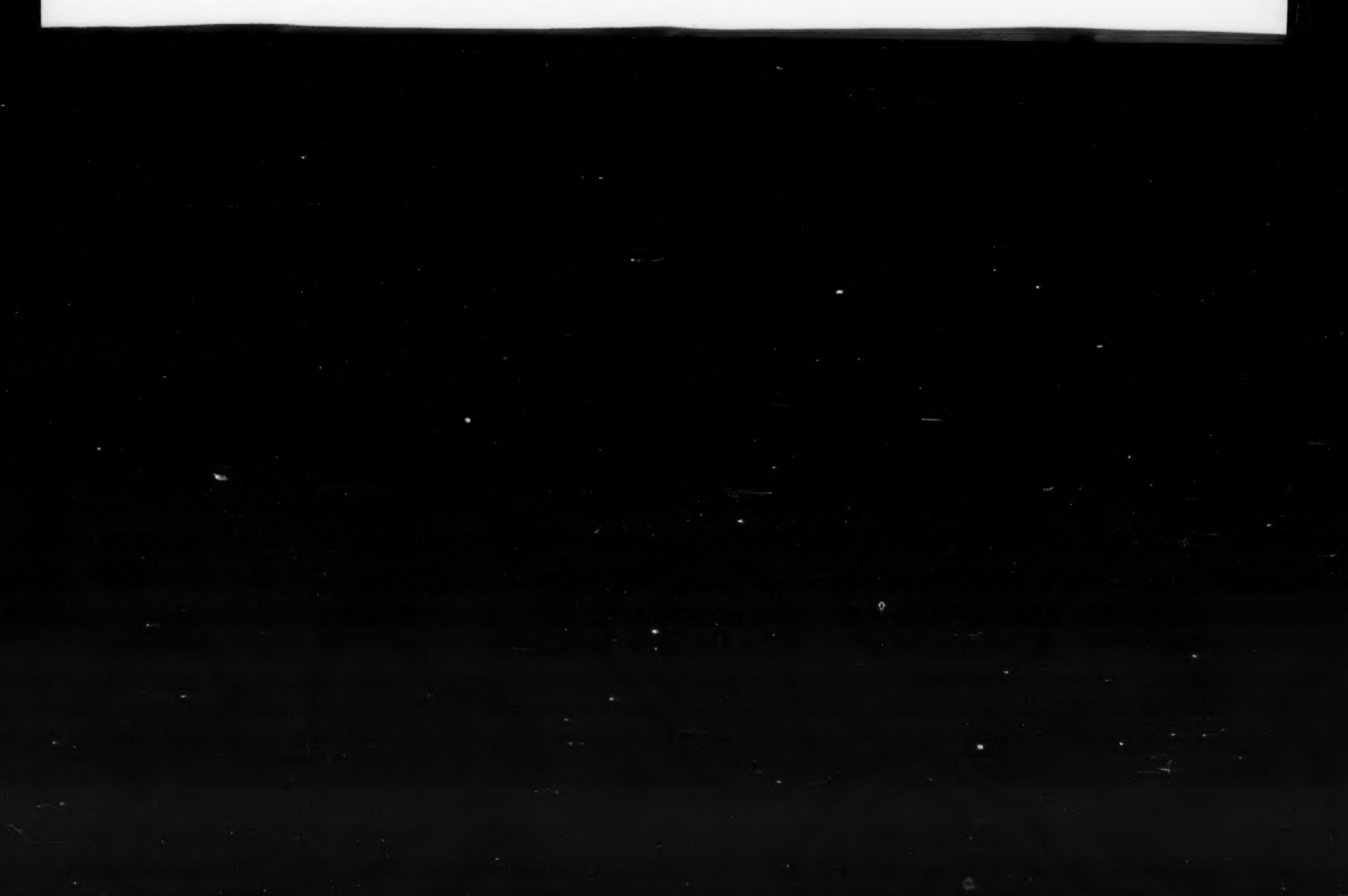
REPRINTS AVAILABLE

Symposium on Forage Evaluation

A combined reprint of the eight papers on Forage Evaluation, given at the Intersociety Symposium at Purdue University, August, 1958, and appearing in the Agronomy Journal, Vol. 51, April, 1959, are available. These reprints, at 80 cents each, may be ordered from the American Society of Agronomy, Central Office, 2702 Monroe Street, Madison 5, Wisconsin.

JOURNALS NEEDED

The May, June, and September, 1958, issues of this Journal are needed for orders, subscriptions, and late memberships. Send them to the Garrard Press, 510 North Hickory Street, Champaign, Illinois. The Association will pay $0.50 plus postage for each copy.



JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

E. O. HERREID, Editor-in-Chief, University of Illinois
Editorial Address: Station A, Box 718, Champaign, Illinois
Phone: FLeetwood 6-6323

EDITORIAL BOARD

F. J. DOAN, University Park, Pa.
N. L. JACOBSON, Ames, Ia.
STUART PATTON, University Park, Pa.
J. E. LEGATES, Raleigh, N. C.
H. D. EATON, Storrs, Conn.
ROBERT JENNESS, St. Paul, Minn.
J. T. REID, Ithaca, N. Y.
N. L. VANDEMARK, Urbana, Ill.
R. E. ELY, Washington, D. C.
J. J. JEZESKI, St. Paul, Minn.
M. L. SPECK, Raleigh, N. C.
EVERT WALLENFELDT, Madison, Wis.
F. A. KUMMEROW, Urbana, Ill.
DURWARD OLDS, Lexington, Ky.
J. P. MIXNER, Sussex, N. J.
L. M. SMITH, Davis, Calif.

COMMITTEE ON JOURNAL MANAGEMENT

J. T. REID, Chairman
Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

F. J. DOAN
J. V. CAVANAUGH
E. O. HERREID (ex-officio)
H. F. JUDKINS (ex-officio)

OFFICERS OF THE ASSOCIATION

A. C. FAY, President
1245 North Bay Shore Drive, Keystone Island, North Miami, Florida
H. F. JUDKINS, Sec.-Treas.
32 Ridgeway Circle, White Plains, N. Y.
R. E. HODGSON, Vice-President
Beltsville, Md.
E. O. HERREID, Journal Editor-in-Chief
Champaign, Ill.

DIRECTORS

E. L. JACK, Davis, Calif.
H. A. HERMAN, Columbia, Mo.
F. J. DOAN, University Park, Pa.
N. P. RALSTON, East Lansing, Mich.
G. H. WISE, Raleigh, N. C.
E. C. SCHEIDENHELM, Lexington, Ky.
K. L. TURK, Ithaca, N. Y.
H. F. JUDKINS (ex-officio)
E. O. HERREID (ex-officio)

ADMINISTRATIVE INFORMATION

Manuscripts

Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, E. O. Herreid, at the address above. See January, 1954, issue, page 103, for information concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

Advertising

Advertising plates, copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the Journal of Dairy Science in care of The Garrard Press, 510-522 North Hickory Street, Champaign, Illinois.

Nonmember Subscriptions

Nonmember subscriptions are $15.00 per volume in the United States and Canada; $15.50 in all other countries, net and postpaid. New subscriptions and renewals begin with the first issue of the current volume. Renewals should be sent in by December 1 of the current year for the following calendar year to avoid a break in the series. Subscriptions should be sent to the Journal of Dairy Science in care of The Garrard Press, 510-522 North Hickory Street, Champaign, Illinois.

Subscriptions for the British Isles and the British Empire should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Street, Covent Garden, London W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert Street, St. Leonards, N.S.W., Australia.

Changes of mailing addresses, post office notices of undeliverable copies, inquiries about copies lost in the mail, and all other matters pertaining to the mailing list for nonmember subscribers should be sent to The Journal of Dairy Science in care of The Garrard Press at the above address. Claims for copies of the Journal of Dairy Science lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

Memberships

Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

Changes of mailing addresses, inquiries about copies lost in the mail, requests for back copies and all other matters pertaining to the mailing list for members of the American Dairy Science Association should be sent to Mr. Judkins at the above address. Claims for copies of the Journal of Dairy Science lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

Form 3579 to be returned to Garrard Press

Printed by The Garrard Press, 510-522 North Hickory Street, Champaign, Illinois. Entered as second-class matter at the post office at Champaign, Illinois, under the act of March 3, 1879.

Copyright, 1959, by the American Dairy Science Association. Printed in the U. S. A.

SOMETHING NEW ON...
MASTITIS

Write to Editor of
The Surge News at

BABSON BROS. CO.

2843 West 19th Street • Chicago 23, Illinois

ATLANTA • DALLAS • KANSAS CITY • MINNEAPOLIS
SACRAMENTO • SEATTLE • SYRACUSE • TORONTO

BOOKLET NOW READY FOR FARM DISTRIBUTION

"MASTITIS IS YOUR PROBLEM" . . . a booklet reprinted from "The Surge News," treats a complicated and scientific subject with solid, practical and understandable words.

Written by George Mather, a man with over 60 years' experience with dairying, this book can bring an important subject to the attention of men most concerned with mastitis prevention and control.

© Babson Bros. Co., 1959

PEOPLE AND EVENTS

COLLEGE AND UNIVERSITY NEWS

P. S. Lucas Retires at M.S.U.

P. S. LUCAS, Professor of Dairy at Michigan State University, has retired after nearly 40 years of service at the Michigan school. He has served the dairy industry in a distinguished and practical way for more than half a century. He will remain in the East Lansing community and will be available as a consultant in the dairy and food industry.

P. S. Lucas

Born in Aurora, Ill., Prof. Lucas graduated from Purdue University in dairy manufactures in 1914. Following graduation he worked as a chemist for the Alamito Dairy Co., Omaha, Neb., 1914-15, and returned to the Indiana Agricultural Experiment Station during 1915-16. He received his M.S. from M.S.U. in 1926.

In 1916 he became an instructor in dairy manufactures at Oregon Agricultural College, was promoted to assistant professor in 1917, and during World War I developed cheese making methods using pepsin as a substitute for Danish rennet which was not available. In 1919 he returned to Purdue where he was promoted to associate professor in 1920.

In 1922 he joined the Michigan State faculty as an associate professor. He has taught all of the dairy courses at M.S.U. but specialized in plant management and ice cream. He has published hundreds of articles, bulletins, and circulars in his field. Prof. Lucas has also served as column editor or contributor to the *Ice Cream Review, Milk Plant Monthly,* the *Journal of the American Public Health Service,* and the JOURNAL OF DAIRY SCIENCE.

From 1923 until 1937 he was manager of the M.S.U. creamery. He was a founder and first secretary of the Detroit Dairy Technology Society, was a member of Phi Kappa Phi, the A.D.S.A., the International Association of Milk and Food Sanitarians, the M.S.U. Friends of the Library, and other Michigan Technical Societies.

During 1958, Prof. and Mrs. Lucas were honored by former students of Prof. Lucas, the Detroit Dairy Technology Society, the Michigan Association of Sanitarians, and M.S.U. officials.

Alexander of Illinois Retires

M. H. ALEXANDER, associate professor of Dairy Science at the University of Illinois, has retired after 16 years on the faculty.

Mr. Alexander graduated from Illinois in 1925. He taught vocational agriculture at Galesburg, Ill., high school for two years before accepting a position at Blackburn College, Carlinville, Ill., in 1927. Except for the time he spent earning an M.S. degree from Illinois in 1934, he taught at Blackburn until he joined the U. of I. staff in 1943.

M. H. Alexander

In 1952, Mr. Alexander was the first U. of I. staff member to take part in the exchange agreement between the University of Illinois and the Allahabad Agricultural Institute in India. During his 18-month stay in India, he taught animal husbandry courses at the Institute and traveled throughout the country studying food production problems.

Mr. Alexander is the author of numerous scientific papers dealing with dairy science. Most of his research has been directed toward the development of pipeline milk systems for dairy farms, a field in which he is an eminent authority.

Letters to the Editor

My heartfelt appreciation to the officers of the American Dairy Science Association for their kind thoughtfulness in sending me, through you, the August issue of the Journal of Dairy Science. I am so glad to have this Journal and will consider it one of my most valued possessions, together with the beautiful note which came with it and the tribute to my husband.

You have all been so good to me, which shall remain such a pleasant memory.

MRS. WILBER J. FRASER

Even though I have retired, my 38 years of membership in the American Dairy Science Association will continue to hold my interest and I hope to be a much better attendant at the Annual Meetings than I have in the past. In fact my retirement is only a partial one. Beginning September 15th I am going to spend several months with Iranian livestock men in

Objective:

TO ASSURE FULL VITAMIN POTENCY AND FLAVOR STABILITY DURING THE SHELF LIFE OF THE MILK

It is important for all those associated with the milk industry to know about the extremely high quality of Vitex® Vitamin Concentrates. There is perhaps no other industry where health and sanitation standards, where inspection, control and regulation, play such a key role. In consequence, it is well to realize that Vitex concentrates meet or exceed every criterion and are entirely acceptable as a food additive.

Vitex concentrates are processed in a Grade A plant under AAA standards. Every batch of pure vitamin crystals is assayed before processing. And not one batch of the finished product can be released for shipment until the laboratory provides a clean bill of health concerning: total plate count; thermophile count (37° and 55°C) at 10 days; viscosity; physical appearance (fat and solids separation at 10 days at 37°C); final report of vitamin assay.

Vitamin additives offer dairies a profitable means of providing better balanced nutrition. Vitex Vitamin Concentrates are the best assurance of purity, potency and stability.

VITEX LABORATORIES

A Division of NOPCO CHEMICAL COMPANY

GENERAL OFFICES: 60 Park Place, Newark, N.J.
PLANTS: Harrison, N.J. • Richmond, California

NOPCO®

Pioneer Producers of a Complete Line of Vitamin Concentrates for the Dairy Industry

this country. Both ICA and FAS have indicated that there will be other such assignments in the future with livestock and extension groups.

At the end of five and one-half years in Iran, we had 16 poultry circulars published and six dairy bulletins. We had livestock specialists trained in every state and a very good livestock organization in the National headquarters at Tehran. There are 13 artificial insemination centers and 8 to 10 subcenters operating. More than 20,000 half-breed Brown Swiss cows were averaging about 1,188 liters more milk, annually, than were the cows of native stock. In terms of American money, the Brown Swiss were returning about $130 more per cow than the native stock. The Iranians told us that every year they make more from the increased milk production than the American Government has spent for the entire livestock program for eight years. This includes veterinary services, poultry, swine, sheep, and goat programs. This same statement can be made for the increased production of poultry products. Where native cockerels have been replaced by the New Hampshire breed of cockerels, tremendous increases in size and number of eggs have been obtained from the half-breed stock.

The Pack Animal program is getting under way through breeding better mules. One mule does more work than four Iranian oxen and does it better. The economic possibilities through saving livestock feed and making more beef available are enormous.

The work on the improvement of native sheep and goats is well under way and on a very sound basis. Eventually it will contribute as much or more to Iranian wealth and morals as any other phase of our work.

Improved feeding has been a very important phase of our work. Of the 650,000 tons of beet pulp in the country, practically none of it was fed before 1952. Now practically all beet pulp is fed except where the distances are too great. However, this is being overcome by the installation of dryers. Great progress has also been made in the development of better pastures and the production of alfalfa.

I must mention also the Veterinary Program which was within my scope of activities. It has made more friends and built the morale and economy of the nation more than any other phase of the Point IV Program. With about 80 trained veterinarians covering the country, the dreaded Anthrax, Foot and Mouth disease, Newcastle's disease, and Sheep pox which used to make whole areas destitute, are now being brought under control.

Without exception it has been the most satisfying six years of my life. If I had not had so many birthdays, I would have been very happy to have acceded to the petition of the Iranian Government and the request of the American mission in Tehran, to return to Iran.

E. M. HARMON
2227 Harden Circle
Hendersonville, North Carolina

NEW from Johnson & Johnson research

RAPID-FLO®

VACULINE®

MILK FILTERS

. . . *engineered* by Johnson & Johnson for in-a-line filters. Available in 4 9/16", 5" and 6" disks; or 2⅜" x 6½" tubes.

Tell your producers with in-a-line filters about new RAPID-FLO VACULINE—*engineered* for safe filtration and clean milk production, plus the extra benefit of a RAPID-FLO® CHECK-UP for mastitis and sediment.

FILTER PRODUCTS DIVISION Johnson & Johnson

4949 WEST 65TH STREET • CHICAGO 38, ILLINOIS

Copyright 1959, Johnson & Johnson, Chicago

Have You Heard What They're Saying About *MATRIX*?

FROM NEW YORK

"Matrix promotes efficiency and improves quality. We are recommending its use to other plants in the district."

FROM CALIFORNIA

"We have started to use Matrix at our cottage cheese plant and plan to have our other cheese plants use Matrix in the future."

Pretested Growth Media for Preparing Perfect Mother Cultures

FROM OHIO

"Matrix has improved the quality of our cottage cheese."

FROM WISCONSIN

"Our continued use of Matrix is ample proof that we have come up with a program that is nothing short of terrific."

FROM PENNSYLVANIA

"We have been using Matrix for several months and like the idea of using a tested and proven powder."

FROM TEXAS

"We have successfully used Matrix for six months — and get much better results."

FROM ILLINOIS

"We think Matrix is a great improvement — makes culture preparing even easier."

ORDER FROM YOUR JOBBER

GALLOWAY-WEST COMPANY, FOND DU LAC, WIS.

Cornell

A.D.S.A. Past President K. L. TURK, who is spending this fall touring Mexico and South America, reports the following news from his travels:

"We have now been in Mexico about five weeks and will be moving on to Colombia the latter part of the month (September). Much of my time has been spent traveling to visit livestock farms and experiment stations. It has been an extremely interesting experience. For example, the past four days have been spent in the Gulf Coast area of Veracruz which furnishes most of the beef supply for Mexico City. It is a tropical region and quite warm, but produces excellent grass for grazing.

"Among many places of interest, I visited a sterilized milk plant in Poza Rica a couple of days ago. The plant is processing about 6,000 liters per day and has a capacity of 20,000 liters. The milk has a cooked taste which I didn't like, but they claim that consumption is increasing. Milk for this plant is produced from mixed herds, mostly of native or Criollo type cattle that have been 'up-graded' with Zebu and Brown Swiss bulls.

"I've seen a number of very good herds of Holsteins at the higher elevations where the climate is more favorable."

Georgia

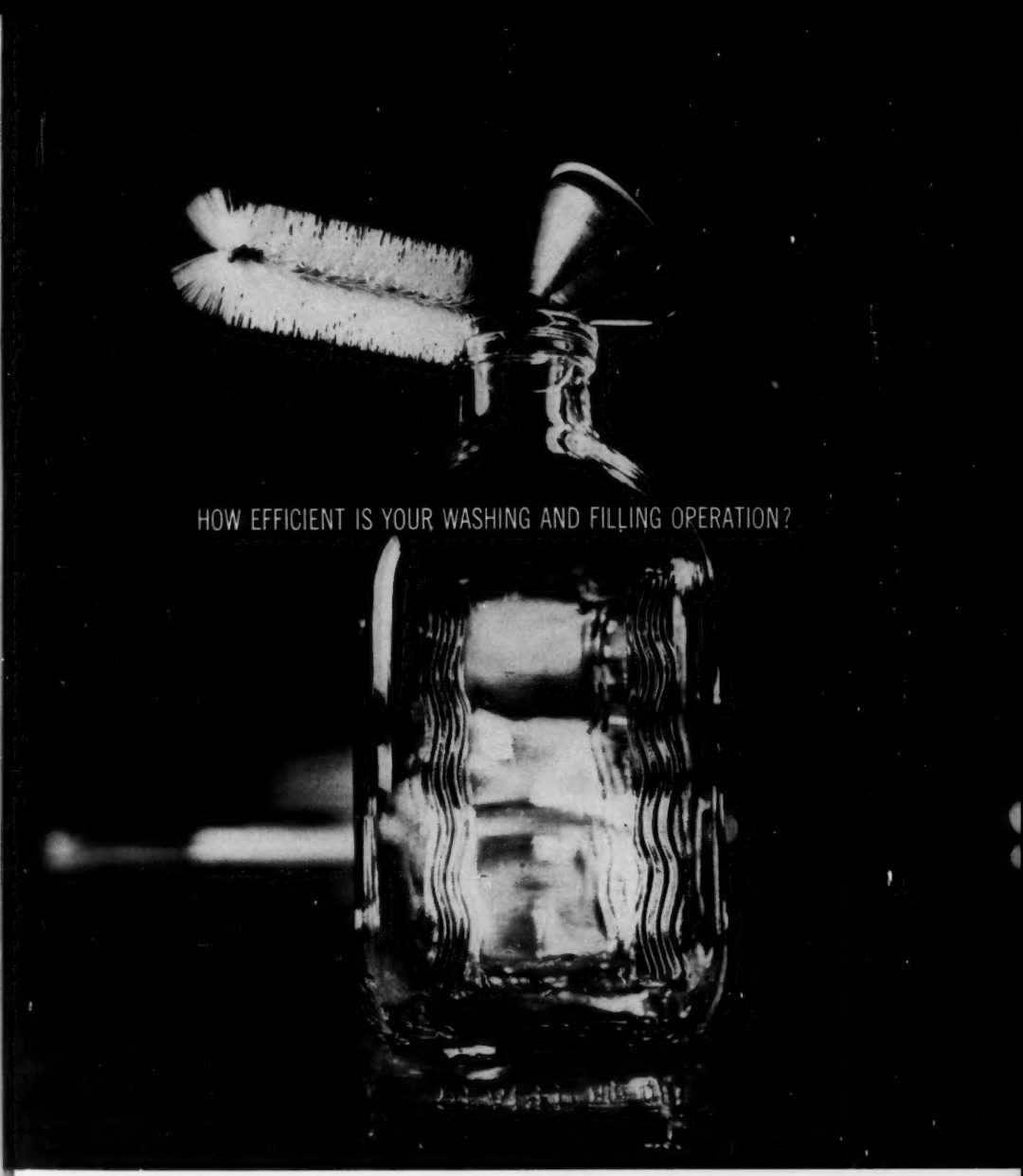
The Sixth Annual Sanitarians Conference and Meeting of the Georgia Chapter of the International Association of Milk and Food Sanitarians was held Sept. 24-25, 1959, at the University of Georgia Center for Continuing Education. A featured speaker was R. V. HUSSONG, Manager, Fundamental Research Laboratory, National Dairy Products Corporation, Glenview, Ill.

Through the courtesy of a local cooperative dairy plant, the annual chicken barbecue for dairy students and their dates and the faculty will be held at the beginning of the Fall Quarter.

Approximately 100 attended an Artificial Breeding Conference for those interested in breeding dairy cattle, held on September 3 at the Rock Eagle 4-H Club Center, Eatonton, Georgia. N. D. BAYLEY, U. S. Agricultural Research Service, Beltsville, Maryland, L. R. SCHULTZ, American Breeders Service, Chicago, and several authorities within the state were on the faculty.

Louisiana

The second annual sale of artificially sired heifers, held on July 18 at Franklinton in connection with the Louisiana Artificial Breeding Cooperative meeting, averaged $311 for 34 heifers. These heifers were bred to freshen this fall.



Profitable Brands Start With Cherry-Burrell

Obsolete equipment can't meet today's efficiency demands. To stay competitive, operators need equipment that reduces man-hours, reduces product and container loss, increases in-plant profits. It is Cherry-Burrell's job to help you do this.

Cherry-Burrell washers wash bottles thoroughly, gently and efficiently. The combination of high soak temperatures and low caustic assures sparkling clean bottles with minimum etching. The exclusive universal pocket gives you the flexibility to handle all bottles from tall half pints through rectangular half gallons — all standard finishes 38mm through 56mm. Cherry-Burrell washers also available in a wide range of sizes and capacities to wash quarts, half-gallons and gallons.

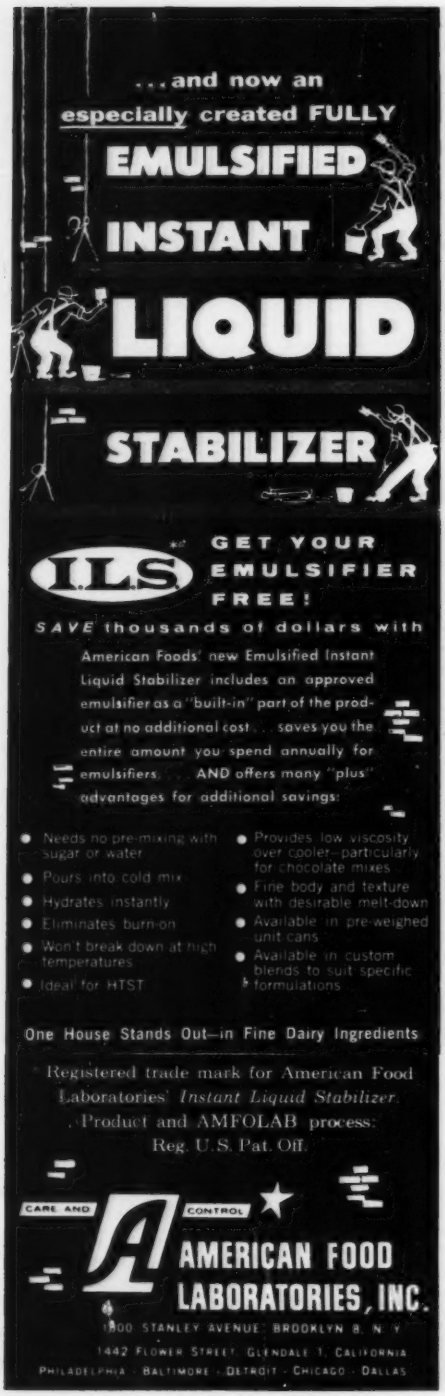
Cherry-Burrell fillers assure trouble free operation, reduce man-hours per unit of product. Accurate filling eliminates product loss. Vacuum type filling eliminates filling of chipped or broken bottles. Fillers available to fill bottles from half-pints to gallon jugs.

Let a Cherry-Burrell Representative show you how a combination of Cherry-Burrell washers and fillers, selected for your specific needs, can increase your profit. Write him today. There's no obligation.

Your one complete source for profit engineered dairy equipment, service and supplies. Sales and Service in 58 cities.

CHERRY-BURRELL
CORPORATION
CEDAR RAPIDS, IOWA

Dairy • Food • Farm • Beverage • Brewing
Chemical • Equipment and Supplies


...and now an
especially created FULLY
EMULSIFIED
INSTANT
LIQUID
STABILIZER
I.L.S.
GET YOUR EMULSIFIER FREE!
SAVE thousands of dollars with
American Foods' new Emulsified Instant Liquid Stabilizer includes an approved emulsifier as a "built-in" part of the product at no additional cost... saves you the entire amount you spend annually for emulsifiers... AND offers many "plus" advantages for additional savings:
Needs no pre-mixing with sugar or water
Pours into cold mix
Hydrates instantly
Eliminates burn-on
Won't break down at high temperatures
Ideal for HTST
Provides low viscosity over cooler—particularly for chocolate mixes
Fine body and texture with desirable melt-down
Available in pre-weighed unit cans
Available in custom blends to suit specific formulations
One House Stands Out—in Fine Dairy Ingredients
Registered trade mark for American Food Laboratories' Instant Liquid Stabilizer. Product and AMFOLAB process: Reg. U.S. Pat. Off.
CARE AND
CONTROL
AMERICAN FOOD LABORATORIES, INC.
1800 STANLEY AVENUE · BROOKLYN 8, N. Y.
1442 FLOWER STREET · GLENDALE 1, CALIFORNIA
PHILADELPHIA · BALTIMORE · DETROIT · CHICAGO · DALLAS

Sixteen Holstein heifers averaged $347. The highest price, $440, was paid for a registered heifer consigned by the Louisiana State University. Four grade Holsteins sold for an average of $405.

Nine Guernsey heifers averaged $275. The top price, $350, was paid for a grade.

The Jersey heifers, nine in all, brought an average of $278. A registered animal consigned by the Southeast Louisiana Experiment Station at Franklinton, sold for $370. A grade Jersey also brought $370.

The relatively high prices paid for the grade animals were due to the fact that all of these heifers were sired by bulls owned by the Louisiana Artificial Breeding Cooperative and were virtually up to the level of pure-breds for commercial milk production.

Michigan

C. A. LASSITER has been named head of the Dept. of Dairy at Michigan State University.

A native of Kentucky, Mr. Lassiter graduated from the University of Kentucky, Lexington, in 1949, and received his M.S. degree in 1950. He then took graduate work at Michigan State University during 1950-52 in the field of dairy cattle nutrition. In 1952, he returned to Kentucky to teach and do research, a position he held until 1956.

He returned to Michigan State in 1956 where he taught and did research until his appointment as department head this past June.

Mr. Lassiter is a recipient of the Feed Manufacturer's Award in 1958.

Nebraska

CHRIS SANDERS, 45, agricultural representative for the Cooper Foundation and University of Nebraska dairy extension associate, died recently after five months of illness. Mr. Sanders initiated and directed the Cooper Foundation Dairy Calf program which sponsored an annual nonprofit sale of purebred dairy calves to 4-H and F.F.A. members. The Cooper Foundation also cosponsored the eight Junior District Dairy Shows in Nebraska and the annual 4-H dairy club leaders training program.

Mr. Sanders served for a number of years as secretary of The Nebraska Brown Swiss Association, was an official classifier for the National Association and had judged Brown Swiss at a number of State Fairs. He served for a number of years as an assistant superintendent of dairy cattle at the Dairy Cattle Congress in Waterloo, Iowa, and was a member of the Dairy Shrine Club.

In Lincoln, Mr. Sanders was a member of the Capital City Kiwanis Club, American Legion, and St. Teresa's Catholic Church. He was a veteran of World War II.

Lactose, Edible—pure sugar from milk

Only one sugar offers you such a VARIETY of profitable applications

Discover for yourself the product-improving, profit-making potential of Lactose, Edible, in the dairy field. Seldom do you find such a versatile material.

For example, Lactose (pure milk sugar) adds "old-fashioned" goodness and body smoothness to buttermilk. Lactose builds a definite, more pleasing, flavor identity in skim milk—eliminates the chalky, powdery taste and staleness often associated with ordinary modified skim milks. In chocolate drink, Lactose makes possible a 50% savings in butterfat content with no sacrifice in flavor or quality. Lactose also improves the quality of cottage cheese dressing.

Only Western can supply Lactose, Edible, in a full range of particle sizes. Strict chemical and bacteriological specifications, rigid quality control and years of experience assure top performance.

Find out how Lactose can help you. For complete information and product samples, write our Technical Service (state applications under consideration). Address: Department 57K.

Lactose

WESTERN CONDENSING COMPANY

Appleton, Wisconsin

World-Wide Supplier of High-Quality Milk Derivatives

Distributed Nationally by
CHEMICAL DEPARTMENT,
McKESSON & ROBBINS, INC.
60 conveniently located warehouses

Oklahoma

Dr. S. D. MUSGRAVE has been named professor and head of the Dept. of Dairying at Oklahoma State University.

Musgrave, 40, has been on the OSU dairy department staff since 1951. Farm-reared in Crawford County, Ill., he attended Blackburn College, Carlinville, Illinois, and the University of Illinois, graduating from the latter university with a B.S. degree in dairying in 1947 and an M.S., also in dairying, in 1948. He received the Ph.D. degree in animal breeding and nutrition and veterinary physiology at Cornell University in 1951. He studied at North Carolina State College for six months in 1958.

S. D. Musgrave

He is vice-chairman of the A.D.S.A. production division. He is also a member of the American Association for Advancement of Science, American Society of Animal Production, American Genetics Association, and Sigma XI science fraternity.

During World War II Dr. Musgrave served for five years in the U. S. Army, including European service, and he is a reserve officer in the Medical Service Corps.

Completed Theses

Ph.D. Degree

L. A. JOHNSON—Heritability and repeatability of first, second, third, and fourth records of varying duration in Brown Swiss cattle. Michigan State University, East Lansing.

E. M. MIKOLAJCIK—Physiology of oxytetracycline resistant and sensitive *streptoccocus lactis*. Ohio State University, Columbus.

M.S. Degree

G. C. KRESHECK—The acid phosphatase enzyme system of milk. Ohio State University, Columbus.

D. R. BOWDEN—A comparison of zero, low, and high level grain feeding to lactating cows on permanent, improved, and supplemental pastures. University of Kentucky, Lexington.

J. P. BATES, JR.—A comparison of feeding separately corn cobs and a liquid urea-molasses-mineral mix with and without ethyl alcohol of yearling dairy heifers. University of Kentucky, Lexington.

B. F. BROWN—A comparison of zero, five, and ten pound levels of grain feeding to lactating

Wiley BOOKS

First suitable text in English to deal with . . .

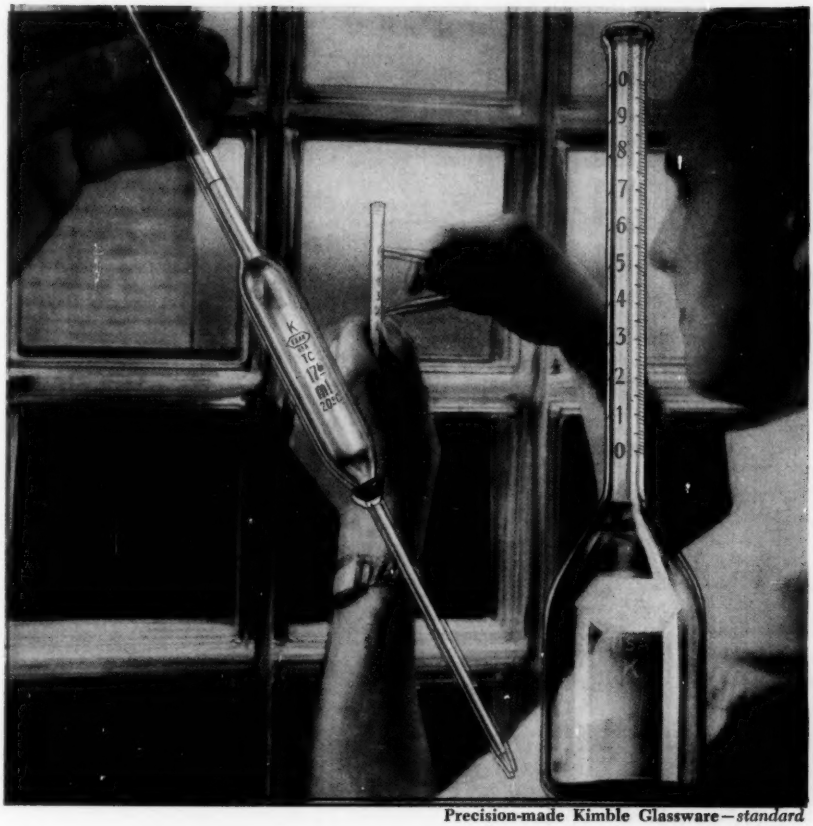
PRINCIPLES OF DAIRY CHEMISTRY

By ROBERT JENNESS, *Professor of Agricultural Biochemistry, University o Minnesota; and* STUART PATTON, *Associate Professor of Dairy Science, Pennsylvania State University.* This is the first book written in the English language designed to meet the present needs of technologists specializing in the principles of dairy chemistry. Main emphasis is directed toward understanding the nature of milk—the chemistry of milk constituents—the interaction of constituents with one another under various conditions—and the relationship of all these facts to the production and storage of dairy products.

Other sections describe the principles of analyses and tests on milk, milk constituents and milk products. The authors go one step further than most books, articles and laboratory manuals on the subject and explain the physical-chemical principles involved and the various merits and limitations of such procedures. Suitable references are given where complete details are desired.

1959. 446 pages. Illustrated. $8.75. Write for your examination copy today.

John Wiley & Sons, Inc. 440 Fourth Avenue, New York 16, New York



Precision-made Kimble Glassware—*standard in an exacting industry.*

DURABLE!

KIMBLE DAIRY TEST WARE

Their long, useful life means low costs for you

Careful fabrication from uniform machine-made tubing and hand blown blanks; durable, easily readable calibration scales; uniform product design; and individual retesting for accuracy make Kimble Dairy Glassware a standard for long, dependable service—careful tempering to equalize strains creates a product of maximum strength and longest life expectancy.

Outstanding quality and assured accuracy have established Kimble Dairy Glassware as a recognized standard of the dairy industry—for research and control. It meets all federal and state requirements—passes the most rigid tests for accuracy and serviceability. Kimble Dairy Glassware is distributed by leading dairy supply houses throughout the United States and Canada. Or write Kimble Glass Company, subsidiary of Owens-Illinois, Toledo 1, Ohio.

KIMBLE DAIRY TEST WARE
AN (O) PRODUCT

OWENS-ILLINOIS
GENERAL OFFICES • TOLEDO 1, OHIO

dairy cows on permanent, improved, and supplemental pastures. University of Kentucky, Lexington.

G. W. BROWN—The efficiency of utilization of urea by growing dairy heifers. University of Kentucky, Lexington.

D. W. CLAYPOOL—The effect of minor changes in the ingredient composition of the ration on rumen microbial activity. University of Kentucky, Lexington.

C. E. GRAVES—The effect of certain vacuum treatments on the flavor and physical characteristics of fluid milk. University of Kentucky, Lexington.

MELVIN HOEFLIN—A comparison of the Cenco and O'Haus methods with the Mojonnier method for determining total solids of dairy products. University of Georgia, Athens.

J. S. CONNER—An economic analysis of alternate methods of assembling and receiving milk for selected Georgia milk plants in 1958. University of Georgia, Athens.

J. K. MILLER—A comparison of feeding grass silage, spring pasture, and a combination to dairy cows. University of Georgia, Athens.

H. W. WARBURTON—An economic analysis of Georgia market milk cooperatives. University of Georgia, Athens.

BUSINESS AND INDUSTRY NEWS

Cherry-Burrell

L. R. Hermann

L. R. HERMANN has been appointed director of production for the Cherry-Burrell Corp. He will assume full responsibility for all manufacturing and product engineering activities of the Cedar Rapids and Little Falls Divisions. He also assumes the responsibilities of the division manager at Cedar Rapids.

He has been with the company 38 years, 30 of which have been in various production and manufacturing capacities. In 1952, he was appointed assistant to the president; and since 1958, his duties have included those of director of planning.

Two field sales representatives have joined the Corporation at its Detroit, Mich., sales branch. They are W. S. TOBIN and E. V. MANES.

An Important McGraw-Hill Book . . .

DAIRY PLANT MANAGEMENT

By Paul H. Tracy, *University of Illinois*, George D. Armerding, *Mojonnier Brothers Co.*, and Harold W. Hannah, *University of Illinois*. With the assistance of George W. Harper, Russell M. Nolen, T. G. Stitts, Carl T. E. Sutherland, and G. H. Wilster.

McGraw-Hill Publications in the Agricultural Sciences
430 pages, $8.50

This book was written primarily for college students majoring in dairy technology and for those in industry who may wish to prepare themselves for advancement in the field of dairy plant management. All aspects of the subject are covered, including plant design, equipment purchase, selection of personnel, accident prevention, the role of law in dairy plant management, and advertising. Major emphasis is placed upon labor relations, business management, business law, and plant efficiency.

Send for Your On-approval Copy

McGRAW-HILL BOOK COMPANY, Inc.
330 West 42nd St. New York 36, N. Y.

KLENZADE "BREAKS THROUGH" WITH NEW TYPE CRYSTAL CLEAR CHLORINATED MANUAL DETERGENT

A Remarkable NEW Product . . . with Even More Remarkable Properties

Klenzade Chlorinated Kleer-Mor makes obsolete all chlorinated cleaners. New in conception . . . new in technology . . . new in approach . . . chlorinated Kleer-Mor is based on the recent Klenzade-perfected principle of the use of ORGANIC chlorine to fortify detergency. Out-dated basic phosphates have been entirely eliminated. Chlorinated Kleer-Mor will handle even the hardest of water supplies. For a revelation in speed, penetration, stability, and over-all cleaning power, use chlorinated Kleer-Mor. Packed in new, water-resistant light weight "KLENZ-PACK" with self-contained protective cover. Easily opened with teartape. Here is the first step in the new Klenzade total sanitation program that will enable you to process milk and dairy products of superior flavor and freshness with surprisingly low counts. Klenzade is aiming at a shelf-life of up to 3 weeks or more. May we help YOU?

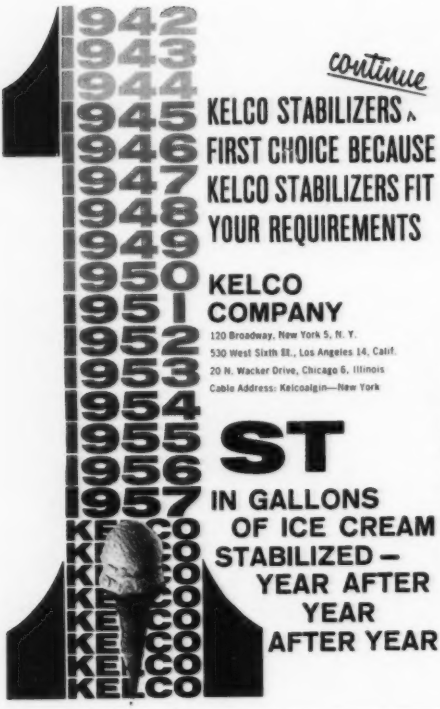
WHY IS Chlorinated KLEER-MOR DIFFERENT?

It's chlorinated with exclusive organic chlorine
Contains no out-dated tri-sodium phosphate
It sparkles . . . crystal clear . . . instantly soluble
Exceptional water conditioner —will handle all water conditions
Foam-stabilized with high wetting speed
Extraordinary detergency, yet moderately alkaline for hand use

ASK TO SEE A KLENZADE REPRESENTATIVE

KLENZADE PRODUCTS, INC., BELOIT, WISCONSIN

KLENZADE PRODUCTS, INC., Beloit, Wisconsin
☐ Please send further information on Klenzade chlorinated Kleer-Mor
☐ Please have representative call
Individual _____ Title _____
Company _____
Address _____
City _____ Zone _____ State _____



Mr. Tobin's business experience has been with Murray Corporation of America, Borden Ice Cream Co., and Penberthy Mfg. Co., all of Detroit, for a total of six years. He attended the University of Detroit where he studied chemistry and mechanical engineering.

Mr. Manes brings to the Corporation 20 years of experience. He has been associated with Oakes and Burger of Ohio, Inc., and the Isaly Dairy Co., both of Youngstown, O. He also studied chemistry and mathematics at the American University, Biarritz, France.

Borden's

M. F. McNeill has been appointed special sales respresentative for the South Atlantic District, concentrating on institutional and industrial sales.

Two ice cream plant managers have also been appointed. They are E. R. Barnette, former manager of the Hickory, N. C. plant, and J. W. Beach, former territory salesman working out of the Hickory plant. Mr. Barnette is new manager for the Charlotte, N. C., plant; and he will retain supervisory responsibility for the Hickory and Asheville, N. C., plants. Mr. Beach will succeed Mr. Barnette at Hickory.

E. J. Franks has been appointed district controller for the Company's New York Fluid Milk District. Mr. Franks has been with Bor-

These are typical comments . . .

"Good Training, Not Like a Convention."

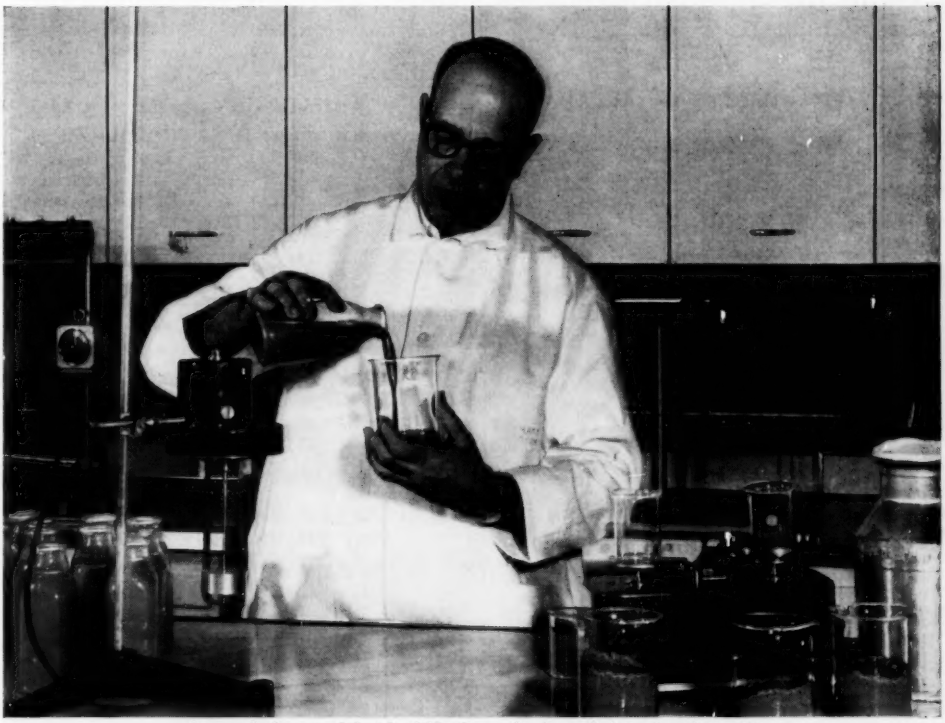
"Saved $12,000.00 With a Clinic Idea."

Come and see for yourself. Share in the collective knowledge and ideas of the progressive men in your field at the 1960 ICE CREAM CLINIC. For reservations write:

G. P. Gundlach & Company

P. O. BOX A, STATION N CINCINNATI 3, OHIO

Gundlach's
1960
Ice Cream
Work Clinic
January 19, 20, 21
Cincinnati, Ohio



A sample from each batch of Kraft powder is made into chocolate milk and performance-tested before any of the batch is shipped to dairies.

This is how we make Kraft Chocolate Flavored Powder so uniformly good!

1. We start with top-grade ingredients. Kraft buys only the finest grade cocoa and quality sucrose. Because these ingredients must meet our rigid specifications, you can count on uniformity of color and flavor in the finished product.

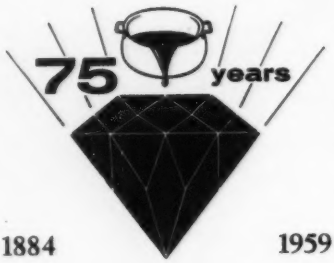
2. We make our own stabilizer. Kraft extracts the stabilizer we use from Irish Moss which we harvest ourselves. Our special process assures thorough suspension with the viscosity you desire. You order the formula designed for your system and its high safety range allows adjustments in sweetness and strength without upsetting balance.

3. We manufacture in small batches. Unlike other manufacturers, Kraft makes chocolate powder only in small batches. This permits more exact control over quality. A sample from each batch of Kraft's powder is made into chocolate milk and its performance tested before any of the batch is shipped to dairies.

Samples for classroom work made available to accredited dairy colleges. Just write your nearest Kraft division office.

KRAFT FOODS—500 Peshtigo Court, Chicago 90, Illinois
Division Offices: New York—Garland, Texas—Chicago—San Francisco

for 75 years
producers of truly
fine chocolate
products



1884 1959

WILBUR
CHOCOLATE CO.
LITITZ, PENNA.

den's for 30 years. He started as a bottling plant worker. In 1946 he joined Borden's Farm Products, and in 1951 became its assistant controller.

The Nestlé Company

C. K. Clausen

C. K. CLAUSEN has been named to the newly created position of manager of trade relations. The appointment is part of Nestlé's new over-all marketing program and is aimed toward closer communication particularly in the area of the discussion of trade policies which are practical and mutually beneficial.

Mr. Clausen has been with Nestlé for more than 40 years in both traffic and sales capacities, most recently as New York Regional Sales Manager.

DAIRY TECHNOLOGY SOCIETIES

Central Illinois—The first fall meeting of the Society was held Sept. 9 in Lincoln. BRUCE LARSON, Dept. of Dairy Science, University of Illinois. His topic was "The Truth About Strontium 90 and Milk." MERLE ORDING, Klenzade

FOR TECHNICAL HELP . . .

call the man from Blumenthal

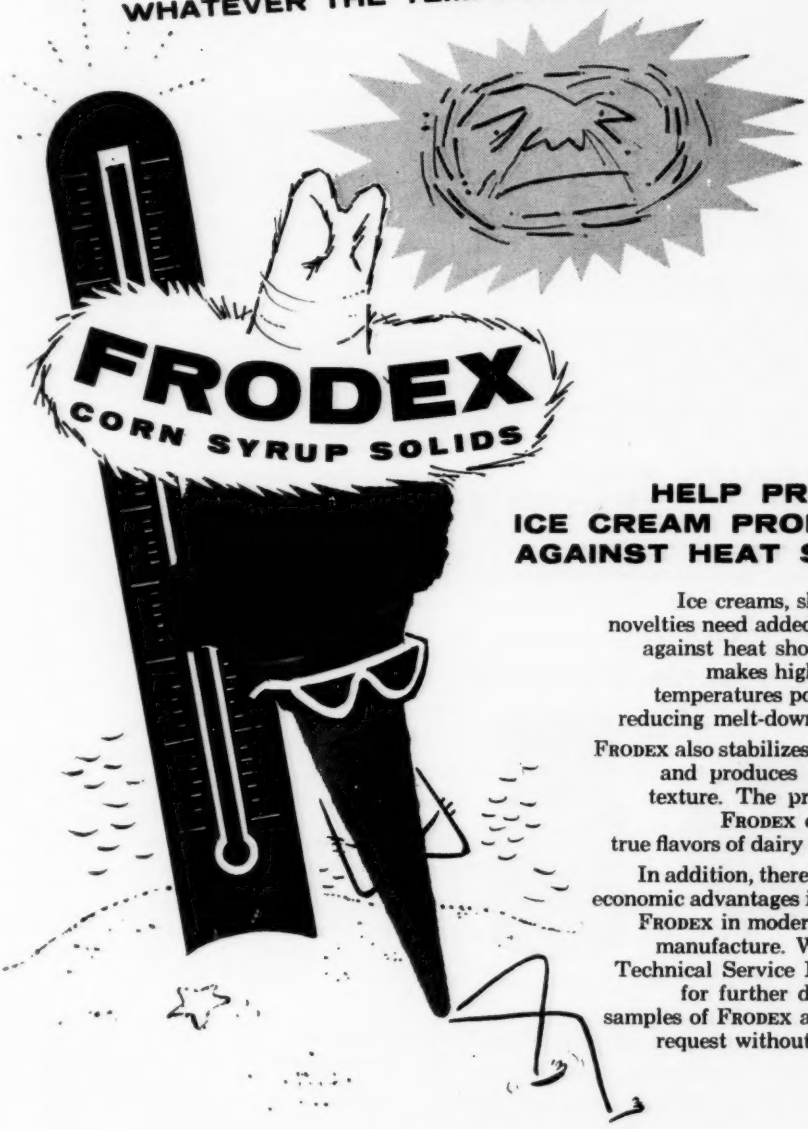
In addition to supplying you with high quality chocolate flavorings and coatings for your ice cream, The Man From Blumenthal will gladly:

1. Discuss prices and prevailing market conditions.
2. Arrange for his laboratory to perform special services that can only be handled by a lab staffed with chocolate technicians and equipped with the latest analytical and pilot plant equipment to produce special types of chocolate for ice cream.
3. Refer your problems to one of our consultants specializing in technical ice cream problems.
4. Conduct research on new ideas suggested by you.

BB

BLUMENTHAL BROS. CHOCOLATE CO.
MARGARET AND JAMES STS., PHILADELPHIA 37, PA.

WHATEVER THE TEMPERATURE . . .



HELP PROTECT ICE CREAM PRODUCTS AGAINST HEAT SHOCK

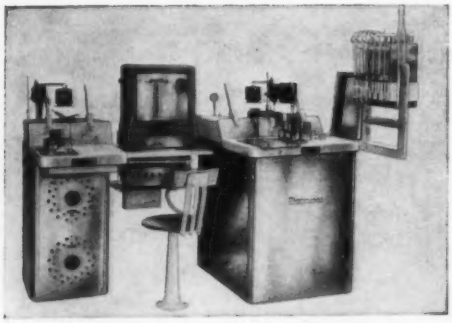
Ice creams, sherbets and novelties need added protection against heat shock. FRODEX makes higher freezing temperatures possible, thus reducing melt-down problems.

FRODEX also stabilizes ice crystals and produces a smoother texture. The pro-sugars in FRODEX enhance the true flavors of dairy ingredients.

In addition, there are definite economic advantages in the use of FRODEX in modern ice cream manufacture. Write to our Technical Service Department for further details. Free samples of FRODEX are yours on request without obligation.

ATLANTA • BOSTON • BROOKLYN • BUFFALO • CHICAGO
DENVER • FORT WORTH • GRAND RAPIDS • LOS ANGELES
MEMPHIS • PHILADELPHIA • PITTSBURGH • PORTLAND
ST. LOUIS • ST. PAUL • SAN FRANCISCO • SEATTLE

AMERICAN MAIZE-PRODUCTS COMPANY
250 PARK AVENUE • NEW YORK 17, N. Y.



protect your profits

- Fat determinations accurate to .03 of 1%.
- Total solids tests accurate to 1/10th of 1%.
- Assures uniformity, quality in finished products.
- No previous skill necessary to operate unit.

Send today for **"Cash Value of Accuracy Chart"**

MOJONNIER BROS. CO., 4601 W. OHIO ST., CHICAGO 44, ILLINOIS

Mojonnier QUALITY ENGINEERED FOR ECONOMY **MILK TESTERS**

Standard Throughout the World for over 40 Years

Dependable Products... Backed by Dependable Service Everywhere

How to make

every batch a success

FLAV-O-LAC FLAKES

"Numbered blends" have proved successful for plants all over the country. Fresh culture *every week* of high quality keeps aroma, smoothness, and flavor uniform in fermented milk products.

A DIVISION OF DALARE ASSOCIATES

Write for details in our Culture Booklet

THE DAIRY LABORATORIES

PHILADELPHIA 3, PA.

Branches: New York • Washington

Products, Inc., and WARREN GALLAGHER, Fullerton Dairy, were in charge of local arrangements. The Society sponsors a $250 scholarship for a freshman in Dairy Technology at the University of Illinois.

Central Michigan—The first fall meeting was held Sept. 16 at Saginaw, and the main speaker was FRANK KOVAL, executive secretary of the Michigan Dairy Products Association. His topic was "Dairying and Scenic High Lights in Western Europe."

Kansas City Area—Sept. 24 was the date of the fall meeting, which featured Past Presidents' Night and an observation of the World Dairy Congress through film slides. Speaker was W. H E. REID, Dairy Dept., University of Missouri.

Kansas—W. H. MARTIN, Kansas State University, spoke on the topic, "Some New Developments in the Ice Cream Industry," and RICHARD BASSETTE, also of K.S.U., spoke on "Newer Knowledge of Feed Flavors in Milk." The meeting was held Sept. 14 in the K.S.U. Student Union, Manhattan.

Texas-New Mexico—The first fall meeting was held Sept. 10 at Plainview, Tex. RALPH DURHAM, head of the Dept. of Animal Husbandry, Texas Technological College, was the main speaker. His topic was "Competitive Production Now and in the Future." The next meeting will be held Nov. 16 at Texas Tech.

Tri-Cities—The first fall meeting was held Sept. 8 with D. H. JACOBSEN, research director of the American Dairy Association, as main speaker. Dr. Jacobsen's topic was "Dairy Product Research and Current Health Problems." Headquarters for the Tri-Cities Society is Louisville, Ky.

Tri-State—Sept. 23 was the first fall meeting and it was held at Wheeling, W. Va. Main speaker was ROBERT TERHUNE, Director of Agriculture for the State of Ohio. His topic was "The Most Pressing Need in the Dairy Industry Today."

Western Michigan—Grand Rapids was the scene of the first fall meeting Sept. 21. Speakers were GLYNN MCBRIDE, Dept. of Agricultural Economics, and A. L. RIPPEN, Dept. of Agricultural Engineering, both of Michigan State University. Their topic was "A Progress Report of an Operational Study of Dairy Plant Costs in Michigan."

Projects, Consultation, and Production Control Services in Biochemistry, Chemistry, Bacteriology, Toxicology—Insecticide Testing and Screening.

Write for price schedule.

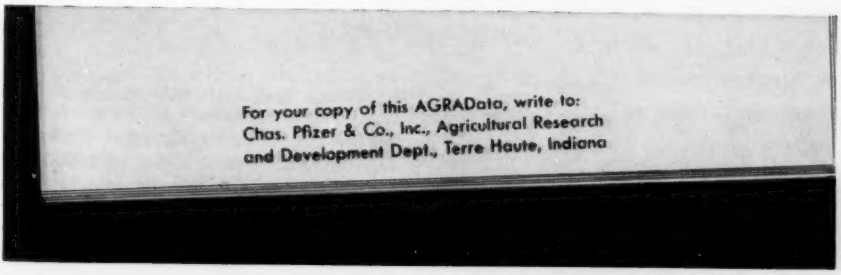
WISCONSIN ALUMNI RESEARCH FOUNDATION

P.O. Box 2217-X, Madison 1, Wisconsin

A Review of Research in Milk Production

Over 125 different papers and research studies contributed to the editing of the latest AGRAData, this issue on milk production. Production in various species is discussed and nutritional requirements are defined, according to research of leading dairy scientists around the world.

In addition to the major feature, each AGRAData includes abstracts on all phases of recent research into animal and poultry nutrition and health. Write for your copy . . . you'll find AGRAData a valuable addition to your file.


For your copy of this AGRAData, write to:
Chas. Pfizer & Co., Inc., Agricultural Research
and Development Dept., Terre Haute, Indiana

NONCHAPPING
IOSAN®
PREVENTS MILK LOSSES
"Stopped mastitis"
I.D., CALIF.

IOSAN helps prevent the spread of mastitis when used on udders and for dipping teat cups. Nonirritating, yet also a powerful cleaner-sanitizer for all equipment. Lowers bacteria counts. Prevents milkstone buildup.

IOSAN is the original patented "Tamed Iodine"® Germicidal Cleaner. Used daily by tens of thousands of milk producers. Especially effective for Bulk Tanks. For a free demonstration contact your regular supplier or **Lazarus Laboratories Inc., Div. West Chemical Products, Inc., 42-16 West St., Long Island City 1, N. Y.**

...Announcing!
NEW PROVEN
DRI-VAC CULTURES
These new Dri-Vac Cultures are the result of over two years research and proving in actual plant operations on various types of cheese and cultured dairy products.
40
41
42
43
Developed and Manufactured by:
CHR. HANSEN'S LABORATORY, INC.
9015 W. Maple St., Milwaukee 14, Wis.

From the Secretary's Desk

Pres. A. C. Fay Confers with Secretary

During this past summer, President and Mrs. A. C. FAY were residing in Tilton, N. H., while your Secretary and his wife were living during August at Boar's Head Inn, Hampton Beach, N. H., where they have spent vacations for over 20 years. This afforded an opportunity for the A.D.S.A. officers to have a conference on a number of future activities of the Association including the formation of new committees.

1960 Membership Campaign

Last year space was provided on the dues notices for members to suggest names and addresses of prospective new members. About 50 new names were suggested and a very good percentage of these became members. With 1960 dues notices now being mailed to nearly 2,600 members, we should get more than 50 prospective members nominated.

H. F. Judkins and A. C. Fay

I hope that all members will give this serious thought and try to think of someone who would be interested in becoming a member of our Association. If in doubt as to whether a candidate is already a member, nominate him anyway. Names will be checked in our files before contact is made.

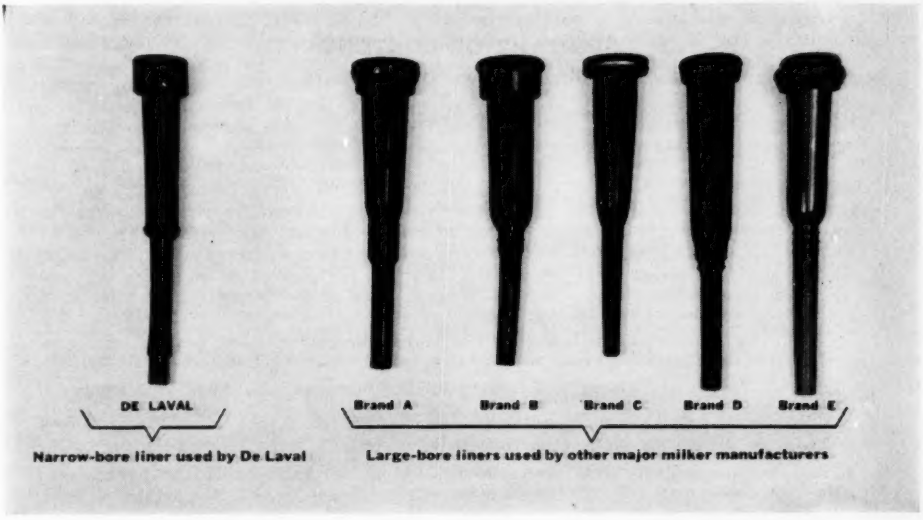
A.D.S.A. Awards Program

The Awards Program of our Association is one of its most important activities. We are fortunate in having a number of organizations so keenly interested in our Association that they will donate these awards year after year. Obviously we owe it to them to make these awards as competitive as possible. It is also important from the standpoint of donors and the winners that the awards go to the very best minds in their particular fields.

Award nomination forms will be mailed to members with 1960 dues notices and it is hoped that more members will make nominations than has been the case in recent years.

H. F. JUDKINS
Secretary-Treasurer

How narrow-bore liners can help prevent mastitis



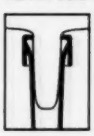
This unretouched photo shows six different makes of teat cup liners. Five are large-bore. That is, the inside of the rubber liner or inflation is large in diameter. *Only the De Laval is narrow-bore.*

Now, recent findings by a California mastitis research team show that narrow-bore liners cause *less* teat injury and udder damage . . . help protect cows from udder injuries that can cause non-specific mastitis, which opens the way to bacterial mastitis. These findings from case histories of over 200 herds also indicate that narrow-bore liners help bring more milk, higher quality milk and reduce the need for antibiotics.

Here's why — The glove-like fit of narrow-bore liners means the vacuum is limited to the end of the teat, allowing blood circulation throughout the teat. The rubber, which is under tension, closely hugs the teat and reduces the impact on the teat end when the liner collapses. What's more, the narrow-bore liner will not crawl up the teat, thus assuring a more thorough milking job and greater comfort to the cow . . . regardless of size of the cow's teats. And because narrow-bore liners *massage the teat,* this helps eliminate tissue stress and udder injury, which often result from the use of other types of liners.

SEE THE DIFFERENCE DE LAVAL NARROW-BORE LINERS MAKE

Large-Bore Liner lets the teat "wobble" around inside the liner, increases impact on teat in liner collapse stage. Can cause udder damage.



Narrow-Bore Liner fits cow's teat like a glove, thus minimizes impact on teat when liner collapses. Reduces danger of udder injury.

DE LAVAL

THE DE LAVAL SEPARATOR COMPANY
Poughkeepsie, New York
5724 N. Pulaski, Chicago 46, Illinois

DE LAVAL PACIFIC COMPANY
201 E. Millbrae Avenue, Millbrae, California

DAIRY INDUSTRY PLANT TRAINING MANUAL

FOR THE INDIVIDUAL AND THE COMPANY

TRAINING FOR LEADERSHIP

Prepared by

THE PERSONNEL TRAINING MANUAL PREPARATION COMMITTEE
THE AMERICAN DAIRY SCIENCE ASSOCIATION

H. B. HENDERSON, University of Georgia, Athens, Georgia, Chairman
F. C. EWBANK, Michigan Milk Producers Association, Imlay City, Michigan
I. A. GOULD, The Ohio State University, Columbus, Ohio
FARNUM GRAY, Southern Dairies, Charlotte, North Carolina
H. F. JUDKINS, American Dairy Science Association, White Plains, N. Y.
H. F. WILLIAMS, Carnation Company, Los Angeles, California

SUMMARY OF CONTENTS

Section I Objectives and Requirements of the Program

Section II Training Program Schedules for Plant Operation

1. Orientation
2. Plant operations
3. Laboratory
4. Engineering and maintenance
5. Procurement
6. Sales and distribution
7. Office work

Schedules providing for varying periods of time are provided for the above categories for the operation of

1. Milk plants
2. Ice cream plants
3. Condensed and dry milk plants
4. Cheese plants
5. Butter plants
6. Evaporated milk plants

Many questions are asked for the trainee to answer before passing from one phase of training to the next and progress reports and rating forms are provided.

Section III Management and Development

1. Developing skills in personnel management
2. Developing skills in quality control and cost

Many study projects are outlined which are not only essential to management development but may result in savings to the plant that will more than pay the cost of training.

Appendix

1. List of professional and trade organizations
2. List of reference books, booklets, and periodicals

COPIES WILL BE NEEDED BY:

Plant Managers
Plant Supervisors
Plant Trainees
Teachers, for a reference text

POSTPAID PRICES:

$4.00 per single copy
$4.00, less 10% for 5 to 9 copies, one order
$4.00, less 15% for ten or more copies, one order

Payment to be sent with order

Ready for distribution November, 1959. It will assist us in deciding on the number of copies to print and insure the earliest delivery possible if you will order **NOW.**

ORDER FORM

H. F. Judkins
32 Ridgeway Circle
White Plains, N. Y.

Date_____

Please find enclosed $_____ for _____ copies of the Dairy Industry Plant Training Manual. Deliver to:

Name_____

Company_____

Address_____

APPLICATIONS AND LIMITATIONS OF QUALITY TESTS FOR MILK AND MILK PRODUCTS. A REVIEW[1]

C. K. JOHNS
Dairy Technology Research Institute, Department of Agriculture, Ottawa, Ontario, Canada

SUMMARY

Changes in milk production and handling practices of recent years have altered the types and behavior of bacteria in milk. There is a need for reappraisal of bacteriological tests currently in use. With growth of bacteria in raw milk virtually eliminated by efficient cooling, tests applied to the freshly taken sample are not always effective in detecting faulty production practices. Encouraging the growth of contaminants prior to testing increases the utility of the tests.

Longer refrigerated storage of pasteurized products is focussing attention on post-pasteurization contamination with psychrophiles. Here again, tests on freshly taken samples are much less useful than those made after these organisms have been encouraged to develop in the product.

Tests for specific groups of bacteria as indices of carelessness in production and processing probably will grow in importance. Coliform tests will have greater usefulness as efficient cooling hinders the growth of these organisms in raw milk, and as their significance in pasteurized milk is more fully appreciated. More emphasis on udder health may also be expected.

The term quality, as applied to milk and its products, embraces a variety of aspects. These include such diverse properties as freedom from dirt, antibiotics, off-flavors, pathogenic organisms, and abnormal numbers of body cells; evidence of cleanliness and care in production and handling as indicated by microbiological analysis; possession of desirable flavor and aroma, and of adequate amounts of those constituents of nutritional importance. Thus the meaning of quality differs from product to product. With milk itself, the bacteriological aspect has received the greatest attention. With butter and cheese, on the other hand, flavor is of far more importance. With ice cream, we are interested in both of these aspects as well as in composition. In dealing with the subject of quality tests, it was considered preferable to take each test in turn and consider its application, where appropriate, to milk and its various products. This review will not attempt to deal with the chemical and nutritional aspects of quality as these are extensive enough to warrant separate treatment.

MICROBIOLOGICAL TESTS

Interest in bacteriological testing of milk stemmed from the discovery that bacteria in milk could cause disease and spoilage. The first systematic investigation of a milk supply was that of Sedgewick and Batchelder in Boston in 1892 (144). From a public health standpoint the importance of bacteriological examinations was quickly recognized and gradually such examinations have become a regular practice. The need (135) for uniform procedures led the American Public Health Association in 1905 to appoint a committee to standardize methods. Studies by this and subsequent committees have resulted in successive editions of

Received for publication June 15, 1959.

[1] Reprints available for 90 days after publication of this paper. For prices refer to page 1434 of the August issue of this JOURNAL.

Standard Methods for the Examination of Dairy Products (2), the 10th edition of which is currently being revised. In Europe there has also been interest in standardizing methods internationally; more recently, the International Dairy Federation has been active in this sphere (77).

With marked advances in the eradication of bovine tuberculosis and brucellosis, together with widespread pasteurization of milk, interest in bacteriological testing of raw milk has largely shifted from the disease aspect. Nowadays, such examinations are more concerned with obtaining an estimate of the degree of care taken in the production and handling of milk on the farm. Nevertheless, it should be remembered that conditions which allow the entrance and multiplication of large numbers of microorganisms may also allow the entrance and multiplication of pathogenic types.

The disease aspect is more important with pasteurized products, however. Recontamination with pathogens can have serious consequences. Direct testing for pathogens is impracticable, but testing to detect any recontamination after processing is now common.

High bacterial populations in milk may result from heavy initial contamination, as from neglected milking machines, from growth in the milk, or from both. Over the years, cooling procedures have greatly improved and, with the farm bulk tank, microbial growth is virtually eliminated. Consequently, cooling may mask careless production practices. This is causing concern to many milk sanitarians and there is a definite feeling that a reappraisal of testing methods and standards is desirable. Attempts to cope with this situation by promulgating stiffer standards—e.g., 50,000 per ml. (36)—are scarcely adequate, and involve setting up two different standards for acceptable milk.

Long ago it was recognized that the more carefully milk is produced, the less important cooling becomes. This is because the udder flora, which comprise the bulk of the bacteria in cleanly produced milk, grows best at body temperature and very slowly at temperatures below 15° C. (59° F.). In Britain, the importance of this has long been recognized. Official procedures (113) for testing raw milk call for it to be held at atmospheric shade temperature (averaging slightly under 60° F.) for 12 or 18 hr. before starting the test. Obviously, this is less satisfactory than a fixed temperature and would be out of the question where wider extremes of temperature are encountered. Recently, it has been suggested (90) that samples be given a preliminary incubation (P.I.) at 12.8° C. (55° F.) for 18 hr. before testing. At this temperature the udder flora fail to multiply. When counts made before and after P.I. are compared, little or no increase is found in carefully produced milks; others, even though initially low, may have increased over one hundred-fold. This procedure thus tends to show up those milks where cooling masks faulty production practices, and rightly puts the emphasis on clean methods rather than on efficient cooling. A somewhat similar procedure has been used with bulk tank milk in Scotland (30).

While bacteriological testing is essential in a quality control program, it is not enough in itself. Regular farm inspection is also considered essential. The work of the fieldman or sanitarian can be made much more effective by an adequate program of bacteriological testing. Both are indispensable.

QUANTITATIVE TESTS FOR TOTAL BACTERIA

The Standard Plate Count (S.P.C.). This method of assessing the bacteriological quality of milk was the first laboratory test devised for this purpose. Before pasteurization came into widespread use, there was a tendency to regard the plate count as a partial index of the freedom of the milk from pathogens. This view has not completely disappeared. Medical authorities in Britain still insist on 37° C. incubation for the plate count method, on the grounds that pathogenic bacteria will be more readily detected at this temperature. (It is usually recognized that the S.P.C. is of no value for this purpose.) Nowadays, it is more generally accepted that the chief value of the standard plate count (S.P.C.) (2) on raw milk is as an indication of the sanitary conditions of production and handling.

Although generally conceded to be the most precise method for assessing the bacterial population, the S.P.C. is not without limitations. No one medium incubated for a short time at a given temperature will bring out all the bacterial types present. Furthermore, colonies may represent single organisms or clumps of hundreds. The degree of experimental error inherent in the method is considerable (175), so that in comparison with chemical tests the plate count is a rather elastic yardstick by which to measure the bacterial content. This, unfortunately, is not always realized by administrators, and unwarranted significance is sometimes attributed to small differences in counts. There is also a tendency to consider the S.P.C. the sole criterion of hygienic quality, and to regard it as the standard against which all other tests for milk quality must be compared. Standardization of plating media, in order to ensure uniform productivity from different batches of media, has yet to be accomplished, although a synthetic reference medium has been developed (131).

Despite these limitations, the S.P.C. is generally conceded to be the most suitable method of examining raw milk with a low (e.g., less than 200,000 per milliliter) bacterial content. As quality improves, it is tending to replace other types of test. Some authorities consider it too laborious and expensive (59, 75); the United States appears to be the only country able to afford it for routine control of raw milk. Even here there is interest in simpler and cheaper procedures, such as the roll-tube method (5, 34, 75) and others (2, 67), especially for unofficial control purposes. In Britain, although the methylene blue test is the official test for routine control (113), the plate count is preferred for advisory purposes (19).

As a result of the studies at the Geneva Agricultural Experiment Station (130), the desirability of an incubation temperature lower than 37° C. gradually has been accepted. Dairy bacteriologists generally prefer 30 or 32° C. for a 48-hr. count. Some would like incubation for 72 hr. for pasteurized products (77). Public health laboratories, on the other hand, have generally preferred the higher temperatures used in their diagnostic work. To try to satisfy both groups, in 1948 incubation either at 32 or at 35° C. was made optional (2). S.P.C. at 32° C. is less likely to disagree with results of dye reduction tests, according to Harris *et al.* (65). Evidence continues to accumulate that 35° C. is too high

for certain types of bacteria (10, 11, 49), but there is still considerable resistance to adopting a lower temperature.

While the plate count is generally conceded to be the most suitable test for measuring the bacteriological quality of pasteurized products, about all it really tells when applied to freshly pasteurized products is how many thermoduric bacteria were present in the milk before pasteurizing. To this extent, a high count is useful in indicating neglected milking equipment, especially milking machines, the chief source of these organisms. In Europe, however, the primary interest appears to be in the keeping quality of the pasteurized milk or cream. Instead of making tests on the fresh samples, it is customary to hold samples at 5° C. for 24 hr., then at 17° C. for another 24 to 48 hr., before testing (16, 18, 125). After such treatment, a coliform test or a methylene blue reduction test is preferred to a plate count as correlating more closely with actual keeping quality at 17° C.

While the same incubation period is specified in *Standard Methods* (2) for both raw and pasteurized milks, the Committee set up by the International Dairy Federation (77) recommended an extra 24 hr. (72 hr.) for pasteurized milk. This is in recognition of the fact that many organisms which survive this treatment exhibit a long lag phase, resulting in failure to form discernible colonies in 48 hr. This is even more true with milk powder samples; after 72 hr., counts are often sharply increased, and colony size is much more satisfactory for counting (86). It is believed that *Standard Methods* should be revised to call for 72-hr. incubation for dry milks; the Committee of the International Dairy Federation (77) has recognized the need and has recommended incubation at 30° C. for five days.

In passing, it should be emphasized that a low standard plate count on a fresh sample is no guarantee of adequate keeping quality (87, 126). Milk may be a week or more old before it is consumed, and during that period has generally been exposed to temperatures which permit the growth of psychrophiles and some mesophiles. A freshly taken sample may show an extremely low S.P.C.—mostly thermodurics—yet, within a week at refrigeration temperature a slight contamination with psychrophiles may result in hundreds of millions per milliliter. As an index of plant sanitation, the S.P.C. made on samples after holding for five days at 7.2° C. (45° F.) is most effective (87); this procedure is much more sensitive than the coliform test, either before or after refrigerated storage. Samples with low counts after such treatment should have excellent shelf-life.

Other cultural methods. The S.P.C. requires considerable glassware, equipment, and media, and is expensive. Consequently, there is considerable interest in simplified methods, even where these may be less precise.

The Burri slant method (2, 28) employs a calibrated loop with which to deposit and spread 0.001 ml. milk over the surface of a hardened agar slope in a test tube. Although less accurate than the S.P.C., it is a useful screening test, especially where, as on the farm, no laboratory facilities are available. Modifications include the oval tube method for determination of thermoduric organisms (2, 119). The "Seeing Is Believing" test of Jamieson (2, 78), an offshoot of

the Burri slant method, can be used to advantage for swab tests on utensils, equipment, etc.

The roll-tube method has been proposed from time to time. With the development of improved apparatus, especially the Astell spinner, interest has revived, and favorable results have been reported (5, 34, 75, 140). The method is said to be used fairly extensively by milk plants in Britain. Heinemann and Rohr (67) have described a simplified procedure employing a loop and a screw-capped bottle which appears to have promise.

The membrane filter (MF) technique for total count yielded lower counts and was more cumbersome to carry out than the plate count method, according to Norwegian workers (57).

Surface plating techniques have been advocated from time to time. The latest of these is that of Mallmann and Broitman (105). None of them appears to have found favor for routine analytical work.

Direct microscopic count. This method (D.M.C.), described by Breed (2, 24) in 1911, has outstanding value in furnishing a bacteriological estimate within a few minutes. It also enables a count to be made of body cells (leucocytes, lymphocytes, etc.); this is especially valuable in indicating mastitis symptoms. With high-count milk the method, in the hands of trained technicians, can often furnish a clue as to whether utensil contamination or growth is responsible, provided the sample is from an individual can. It is also possible to preserve samples for later testing by adding formaldehyde (100). Despite these advantages, its popularity for controlling fluid milk supplies has been declining in recent years. In part, this is due to the trend to lower count levels, especially with farm bulk tank milks, necessitating the examination of many fields for quantitative determinations and, in part, to the realization that the D.M.C. grades high-count milk more leniently than does the S.P.C. (81). Black (21) reported the S.P.C. to give higher counts than the D.M.C., with counts exceeding 50,000 per milliliter; below this level, the reverse was true. The author has confirmed this. Another factor has been the growing recognition that many laboratories using the method lacked trained personnel and adequate equipment, especially illumination (21), and that often far too few fields were being examined (101). The effect of fatigue on qualified, conscientious technicians also has been belatedly recognized (99). The growing difficulty of obtaining suitable personnel, and their reluctance to undertake this work, must also be considered. While the D.M.C. is largely being superseded by the plate count for market milk, it is still a very valuable supplementary test here, and has considerable control value with the higher-count milk used in manufactured dairy products.

While the D.M.C. has been recommended in place of the S.P.C. for the control of pasteurized milk (111, 167), it has not been generally adopted for this purpose. More recently it is coming into use in the control of skimmilk powder (54). Here it gives valuable evidence of the past history of the product not obtainable from the viable count. Much more emphasis on adequate equipment and training and close adherence to *Standard Methods* is essential, however, if the method is to yield reliable results (54).

A modification of the D.M.C. for making counts of thermoduric bacteria was proposed (106), but does not appear to have been adopted. Poor agreement with the plate count has been reported (51).

"Little Plate" method. An attempt was made by Frost (56) to combine the advantages of the plate and direct microscopic counts. His little plate method involved growing the bacteria in a thin layer of nutrient agar on a microscopic slide, incubating in a moist chamber for 8-16 hr., then drying the film, staining and examining under a low-powered objective. The method is cheap and results are available earlier than with the S.P.C. Modifications have been published from time to time, but the method has failed to gain much support. Interest in its use as a screening procedure has recently been reported (44). It has also been proposed for making counts of bacteria, molds, and yeasts in butter (82, 114).

The methylene blue reduction test. The methylene blue reduction test (2), introduced in Denmark and Sweden around 1912, is probably the most extensively used bacteriological test, being widely employed in Europe, Australia, and New Zealand, as well as in North America. Its great advantages are its simplicity, cheapness, reproducibility, and rapid detection of poor-quality milks. Technically trained personnel are not necessary, and several hundred samples can be tested simultaneously. Thus, more frequent testing is possible.

Until recent years there has been good correlation between reduction times and plate counts, especially where the creaming error was minimized by periodical inversion of the dye-milk mixture during incubation. Wilson's (175) extensive studies were so favorable to the modified methylene blue test that in 1937 it officially replaced the plate count for the examination of graded raw milks in England and Wales (113).

Several factors have tended to distort the relationship between counts and reduction times. More productive media and lower incubation temperatures have increased the levels of plate counts. The proportion of thermoduric organisms has increased (152); these are slow reducers. Antibiotics in milk tend to slow down reduction. Psychrophiles, which sometimes comprise a high percentage of the flora (90), fail to grow at 35–37° C. Finally, with more efficient cooling, the bacteria are extremely dormant at the start of the test and substances inhibiting bacterial growth are conserved. The resulting prolonged lag phase so delays reduction that some high-count milks escape detection.

The creaming error, caused by the sweeping of varying proportions of the bacterial population to the surface with the rising fat globules (84, 175), has already been referred to. Wilson (175) recommended inversion of tubes every 30 min., to redistribute the bacteria and thus minimize this error, but hourly inversion is equally effective (83) and more convenient. The value of this practice is greatest with low-count milks; for milks reducing in 3 hr. or less, it is less important (83).

The practice in Britain of aging or storing raw milk samples at atmospheric shade temperature for 12 or 18 hr. before testing has already been mentioned. This stems from the 1920's, when regulations (112) governing the conduct of Clean Milk Competitions called for holding samples for around 24 hr. at atmos-

pheric shade temperature before subjecting them to analysis by the plate count. The weakness of this procedure lies in the wide range of temperatures encountered even in Britain. Thus, although the stipulated reduction time for the summer months is only 4.5 hr. as against 5.5 hr. in winter, summer samples show a much higher percentage of failures to conform than do winter samples. Furthermore, several workers have reported the grading to be too lenient in the winter months, a high percentage of samples with high bacteria counts not being detected (103, 166, 175). Storage at a definite temperature, e.g., 12.8° C. (55° F.) would seem more desirable; during cold weather it would allow the growth of saprophytic contaminants and result in shorter reduction times. Comparison of results from one season to another, and one year to another, also would be facilitated.

On this continent, the methylene blue test is rarely applied to pasteurized milk or cream. In Britain, where the interest is predominantly in keeping quality, Provan and Rowlands (136) recommended that the modified methylene blue or the resazurin test replace the plate count. After being held at 18° C. for 18 to 24 hr., the sample is incubated with the redox indicator at 37° C. for 0.5 hr.; samples failing to reduce the dye by this time are considered to have a keeping time in excess of one day at 18° C. Unfortunately, the official regulations (53) which followed specified holding at atmospheric shade temperature (not exceeding 18.2° C.) in place of 18° C., as recommended; methylene blue, rather than resazurin, was designated as the indicator, although Provan and Rowlands had favored the latter. In Britain, this type of test is still applied to ice cream also, but its suitability appears dubious. As Humphriss (76) states, "After all, the Public Health Officers, the manufacturer and the consumer are concerned with the wholesomeness of the product as eaten, and not after incubation for three-quarters of a day at warm room temperature."

As previously mentioned, some of the more progressive dairies in Europe use the methylene blue reduction test as a measure of keeping quality of pasteurized products (16, 18, 125). Their practice of holding samples at 5° C. for 24 hr. (to facilitate detection of oxidized flavor), then at 17° C. for 24 or 48 hr. before testing, avoids the variation in storage temperature of the British method and allows more satisfactory comparison of results from various periods. However, the coliform test is considered to be superior to the dye reduction test for this purpose (18, 125), particularly since rapid dye reduction is sometimes due to aerobic spore-formers which survive pasteurization (125).

For milk for manufacturing purposes, where standards are not as high as for market milk, the methylene blue reduction test is still widely favored in most countries. Unless and until standards for such milk are made much more stringent, it will doubtless continue in popularity. However, as cooling of such milk becomes more effective, its usefulness in its standard form will probably decline.

The resazurin test. As milk quality improves, reduction time increases, increasing the experimental error and making it difficult to complete testing in the ordinary working day. The introduction of the dye resazurin as a redox indicator to replace methylene blue (132) offered the advantage of earlier indication of Eh change, and aroused considerable interest. This was heightened

by the discovery (137) that resazurin was affected by the presence of excessive numbers of leucocytes etc. and thus could indicate the presence of abnormal (mastitic, late lactation, etc.) milk. This advantage is not possessed by methylene blue and is largely dissipated on prolonged storage. A number of modifications have been described, but only three of these have come into general use.

The 1-hr. test (137), modified from the original test by using 37° C. incubation in place of room temperature, was studied by various workers. In this test the degree of color change from the original blue through pink to complete decolorization is measured. While useful in reflecting abnormal udder conditions, it had limited value except where little quality-improvement work had been attempted; well-cooled milks containing excessive numbers of dormant bacteria often escaped detection (68, 69, 91). Where samples are aged before testing, as is officially required in Britain, this weakness applies only during the colder months. The pink test (84) which followed employed the full pink color as the end-point; this was reached in three-fourths of the time required to reduce methylene blue, but unfortunately the ability to detect abnormal milks was lost. A modification, somewhat more involved, has been proposed by Hempler (69), whereby abnormal secretion may be detected by a reading at 1.5 hr. and bacterial numbers by readings after 3.5 and 5 hr.

The triple reading test (2, 91) was developed to avoid the disadvantages of the 1-hr. and the pink tests. It employs a single end-point (Munsell 5P7/4) with readings after 1, 2, and 3 hr. This end-point is reached in one-half the time required for methylene blue reduction. Reduction beyond this end-point within the first 2 hr., followed by slow subsequent change, suggests reduction by leucocytes. The triple reading test has been recognized as a standard method (2) since 1948, and is widely used in Canada for market milk supplies. In order to distinguish milk of exceptionally good quality from that of only satisfactory quality, Hempler (68) has suggested a slightly stiffer grading with the triple reading test. He comments that the triple reading test, in addition to saving considerable time, "reflects the sanitary quality of the milk on a far broader basis than does the methylene blue test." Overby (127), after a study of various tests for the bacteriological quality of milk, also gave the preference to the triple reading test.

Of recent years, especially where farm bulk tanks are common, there have been criticisms that the resazurin triple reading test fails to detect an appreciable percentage of high-count milks. The organisms are so dormant that reduction is delayed appreciably. Here, too, preliminary incubation (90) is most useful in overcoming this dormancy, as well as in encouraging the growth of saprophytic contaminants. The poor agreement between S.P.C. and resazurin reduction time noted on fresh samples largely disappeared following P.I., and there was close agreement between gradings by these two tests (90). Unfortunately, reduction due to leucocytes is largely dissipated during P.I.; it is necessary to use a direct microscopic examination (2) or the Whiteside Test (117, 171), to detect abnormal milk.

Other forms of the resazurin test are employed in Britain. A 10-min. rejection test is applied to milk of doubtful quality (14). This test was selected during the war years as being the most satisfactory of eight methods compared for detecting milk unsatisfactory for pasteurizing, plate counts being of the order of many millions per milliliter (14). [In other countries, reliance is placed more upon a keen nose on the inspection deck; this appears to be a more delicate test (36).] Where cans are owned by the producer, it is also difficult to visualize cans of milk being held for over 10 min. awaiting results of the test.

In Scotland, a weekly temperature-compensated resazurin test is also officially required (42); the reduction time for acceptable milk varies with the mean atmospheric shade temperature during the preliminary holding period from 2 hr. at 40° F. to 15 min. at 61° F. and over. Where excessive leucocyte activity is suspected, a methylene blue test at 37.5° C. also is run. In Britain, grading is done using a Lovibond tintometer and standard color disks ranging from six for initial color to zero for complete decolorization. This method is considered to be more cumbersome and time-consuming than that using Munsell color standards (85) in test-tubes.

In any modification of the resazurin test, it is well to remember that 8% of the male population is color-blind; also that good illumination, preferably from a daylight type fluorescent lamp (40, 91), is highly important.

Golding's modification (61), wherein dye is added to sterile vials and dried down, is particularly useful for plants lacking adequate laboratory facilities, and for use by fieldmen testing suspected quarters for mastitis (108).

The use of another oxidation-reduction indicator, triphenyltetrazolium chloride, has been suggested (118). Unfortunately, it is extremely sensitive to light. Its usefulness appears to be confined largely to heavily contaminated milks, although it has been advocated for use in the detection of antibiotics and other inhibitory agents in milk (120), and in a keeping quality test for pasteurized milk (26, 41).

QUANTITATIVE TESTS FOR SPECIFIC TYPES OF MICROORGANISMS

Thermoduric bacteria. These organisms are sufficiently heat-resistant to survive ordinary pasteurizing temperatures and thus may be responsible for counts in excess of the legal limit for pasteurized products. They enter milk chiefly from the surfaces of inadequately cleaned milking and handling equipment and thus are an indication of insanitary conditions. Some sanitarians believe the thermoduric count is more useful here than the standard plate count (15). While the thermoduric count is officially determined by plating a suitable dilution of raw milk after being subjected to laboratory pasteurization (2), less expensive modifications have been sought. These include the oval tube method of Myers and Pence (119), streaking measured loopfuls of laboratory pasteurized milk on the surface of poured plates (2); the agar strip method (153), a version of the oval tube method, developed in Britain [it is preferred over the roll-tube method (5), as it requires no special equipment (47); counts by both methods were lower than by the standard plate count, but not enough to invalidate their

successful use]; and inoculating melted agar in a test-tube or small bottle, holding at pasteurizing temperature for 30 min., then spreading the inoculated medium in a thin layer. The Astell version (5) of the roll-tube method has already been mentioned.

Incubation at 32° C. is preferable to that at higher temperatures; 37° C. is too high for some thermoduric species. British workers (37) recommended incubation at 30° C., for four days, but subsequently (47) reduced this to three days. While the results from these simplified methods are more variable than those by the official plating method, they are quite adequate for screening out those milks containing excessive numbers of these organisms. A standard of 10,000 per milliliter, commonly employed, seems unduly lenient.

Thermophilic bacteria. Organisms capable of growing at holder pasteurization temperatures were a serious problem when batch pasteurization was common. With the trend toward higher temperatures with HTST and UHT pasteurization, the interest has diminished. These organisms are detected by incubating plates at 55° C. for 48 hr. (2), by direct microscopic examination of smears (2), or by a dye reduction test (methylene blue or resazurin) incubated at 62–63° C. (94).

Psychrophilic bacteria. For the purpose of this review, psychrophilic bacteria are those able to grow actively at temperatures below 7.2° C. (45° F.). It should not be overlooked, however, that their optimum growth temperatures are generally around 25 to 30° C.; not one of 722 cultures grew at 35.5° C. (49). With milk being held longer at refrigeration temperatures from cow to consumer, opportunities for the growth of psychrophiles have increased greatly. Many of them are proteolytic and lipolytic, and are capable of inducing flavor and other defects in milk and milk products on refrigerated storage.

The current official procedure (2) for counting psychrophiles calls for incubation at 5° C. for seven days. Some claim that this low temperature is too selective. Milk and its products are frequently exposed to temperatures above 5° C.; some species capable of inducing spoilage can grow actively in this range. After seven days at 5° C., colonies are frequently so tiny as to be difficult to recognize; counts after ten days are frequently many times larger, while some workers (23) found 20 days necessary to obtain maximum counts. In Denmark, Hempler (70) prefers 7° C. for ten days;[1] this should be much more satisfactory.

Spoilage in pasteurized milk, cream, and cottage cheese is generally due to psychrophilic growth. Pasteurization destroys these organisms; their presence in processed products represents recontamination. Examination of the fresh sample is not always helpful, since the numbers present may be small (126). A more useful procedure is to hold samples at 7.2° C. (45° F.) for five days, then subject them to the S.P.C. at 32° C. (87). This gives any psychrophiles present an opportunity to grow, and counts will often be well into the millions. This is regarded as a much more satisfactory procedure than making a psychrophilic count on the fresh sample.

[1] This will probably be specified in the forthcoming 11th edition of *Standard Methods*.

Coliform bacteria. The presence of coliform bacteria in milk and its products does not have the same public health significance as does their presence in water supplies. Hence, in routine control work there is no point in attempting to differentiate between so-called fecal and nonfecal types.

For the routine examination of raw milk, interest in North America has been largely confined to certified milk (1), although one state (123) has a standard requiring freedom from coliforms in 1/100 ml. portions. It has generally been felt here that high counts represented growth more than direct contamination. With the widespread adoption of farm bulk tanks, growth will no longer be a factor, and more interest in the use of this test may be expected. The survey of milk supplies in eight U. S. cities reported in 1953 (38) showed some correlation between udder cleanliness and coliform content. The results of this survey (log. average coliform count 5,810 per milliliter) suggest there is room for considerable improvement.

In Britain, various authorities (62, 148, 165) have asserted that the coliform test gives the best indication of cleanliness of milk production; high coliform counts most frequently arise from neglected milking machines (148). The possibility that high counts may come from udders infected with coliform organisms must not be overlooked (164).

In pasteurized products, the use of the coliform test to detect recontamination has been more generally accepted. Stemming largely from the work of McCrady and his coworkers (109), the great value of this test, to both sanitarians and management, has steadily received wider recognition. The excuse that coliforms in pasteurized milk may not be heat-resistant was discredited by Buchbinder and Alff (27); the presence of coliforms in a pasteurized product definitely indicates recontamination and a potential health hazard. Pasteurized products, handled in clean, sanitized equipment, should be entirely free from coliform organisms. That this is possible is shown by the record of the Trifolium Dairy in Copenhagen (125); in 1955, 82.4% of samples were coliform-negative in 1-ml. portions after samples had been held at 17° C. (62.6° F.) for 24 hr. before testing. This procedure is employed by other European dairies. Encouraging results have also been reported by the Quebec Ministry of Health (7), where a limit of 50 coliforms per 100 ml. is in effect. The standard of not more than 10 per milliliter currently in vogue in the United States (168) is so lenient as to be of limited value; the presence of any coliforms should call for immediate investigation.

Two distinct types of test procedure, a plating technique and a tube dilution technique, are officially approved (2) in North America. Divergent opinions have been expressed concerning their relative merits. Where standards are stiff, as in Quebec (< 50 per 100 ml.), the dilution technique is preferred as larger volumes of milk can be tested (7). Where standards are more lenient (168), the plating technique is generally favored. Two shortcomings of the latter are (a) difficulty in deciding whether small red colonies should be counted, and (b) atypical colonies on overcrowded plates.

When applied to products containing other sugars in addition to lactose, e.g., ice cream, positive results must be confirmed to avoid misleading results.

False positive results have been reported where sweetened and unsweetened fresh fruits (bananas, peaches, strawberries) are added to the mix (12).

While 35° C. is currently the accepted incubation temperature on this continent (2), there is evidence that higher counts would be obtained with a lower incubation temperature. In Britain, the Coli-Aerogenes Sub-Committee of the Society of Applied Bacteriology, impressed with the evidence of numerous 37° negative strains, has recommended incubation at 30° C. (139). Belgian workers (174) report that over 50% of strains positive at 30° C. were negative at 35° C.

A unique procedure, the Bacto-strip method (55), has recently been developed for determining coliform counts. A definite volume of milk is absorbed by a sterile strip of filter paper impregnated with a suitable medium; the strip is returned to its plastic pouch and incubated 8–10 hr. The incubation period is inconvenient, and counts are generally lower than those by the regular plating technique (89). The tendency for colonies developing from motile strains to coalesce is a further drawback (60). Nevertheless, the method could be quite useful where no laboratory facilities exist.

The millipore filter technique has been advocated for the determination of coliforms in pasteurized and certified milk (48), but not enough information is available to date to judge of its value here. The use of a nonionic agent to facilitate filtration of 10 ml. of milk or 1 ml. of cream has been suggested (50).

Proteolytic bacteria. Organisms capable of proteolyzing casein are frequently responsible for undesirable flavors in dairy products. Surface taint of butter caused by *Pseudomonas putrefaciens* (43) is an example. The presence of most proteolytic organisms can be detected by plating on an agar medium containing 5–10% sterile skimmilk, and incubating at approximately 21° C. for three or more days. Colonies arising from proteolytic types will be surrounded by clear zones, where the protein has been digested by the enzymes they have secreted. A clear zone may also surround certain nonproteolytic bacteria which produce slight acidity; by flooding the plate with dilute acids, these zones around nonproteolytic colonies will disappear.

Lipolytic bacteria. Organisms attacking fats are often also proteolytic and also psychrophiles. This makes them particularly important in butter, cream, and cottage cheese, which are frequently held refrigerated for extended periods. The free fatty acids liberated from fats by hydrolysis contribute pungent flavor and odor to the product; these may be desirable in certain cheeses, but in other products they generally cause defects.

The enumeration of lipolytic bacteria is based upon detection of free fatty acids liberated from fat added to the culture medium; Nile blue sulfate is the indicator normally used. It stains normal fat globules pink; when the fat is hydrolyzed by lipolytic colonies, the globules stain blue. Care must be taken to avoid the toxic effect of an excessive amount of dye (96). One per cent of sterilized stained fat is added to the tempered medium just before pouring; the fat should be in the form of a fine emulsion. Tributyrin is frequently preferred to natural fat as it is more easily hydrolyzed; however, not all organisms hydrolyzing tributryrin also hydrolyze butterfat.

Molds and yeasts. Proper pasteurization of milk or cream destroys molds and yeasts; thus, their presence in a product indicates recontamination (22). Mold and yeast counts on butter are commonly run to check on plant sanitation; trouble with mold growth on butter is much less frequent when counts are low. The medium currently accepted here as standard (2) is potato dextrose agar, acidified to pH 3.5 before pouring to inhibit bacterial growth. Olsen and Bonner (124) recently reported 100 p.p.m. aureomycin even more useful for this purpose, yeast counts frequently being much higher than with the standard medium. Unpublished results from the writer's laboratory have confirmed this.

Mold and yeast counts are also employed by progressive cottage cheese manufacturers and sanitarians as indices of plant sanitation. A standard of not over 10 per milliliter is currently being met in some areas.

Bacto-strips (55) are also available for determining the mold and yeast count on equipment surfaces, in the air, etc. Good agreement with results from the conventional technique for determining air-borne contamination has been reported (89).

Mold mycelia count. When cream is stored on the farm at unsuitable temperatures for too long periods, growth of *Geotrichum candidum* takes place. Although the mold is killed by pasteurization, dead mycelia pass into the butter in appreciable amounts. They can be counted by a method (2) similar to that used to detect mold filaments in tomato products. This method can, therefore, tell much more about the lack of care the cream has been subjected to than can a viable count on the butter; it is frequently employed by food and drug officials and may be used as the basis for seizure and confiscation of butter.

Fermentation test. Milk for cheese-making is often tested by the methylene blue test, then incubation continued overnight in the fermentation test (153). It is believed by some that the type of curd formed indicates the types of bacteria present in the milk, particularly those causing gassy defects in cheese-making. However, the more commonly held view is that it is better to discriminate against milk heavily contaminated with bacteria; with lower-count levels the lactic acid bacteria in the starter have little difficulty in establishing dominance.

Keeping-quality tests. In Britain considerable emphasis is still placed upon keeping-quality tests for raw milk. Since almost all milk is pasteurized, and since there is usually little correlation between keeping quality before and after pasteurization, this interest seems rather archaic. Various methods have been tried out; of these, the clot-on-boiling (C.O.B.) test (45) is considered the most accurate, although it reflects chiefly souring. In this test, portions of the sample are maintained at 18° C.; morning and evening a fresh portion is immersed in boiling water and examined for clotting; this continues until clotting occurs. The method appears too cumbersome and time-consuming for routine testing.

As already indicated, the dye reduction tests are used to assess probable keeping quality of pasteurized milks in Europe. In the United States, TTC (26) and neotetrazolium (41) have been suggested for this purpose. In Europe, the coliform test after preliminary incubation is considered to be a more reliable

indication of keeping quality (18, 126). In all of these procedures, the pasteurized sample is maintained at a temperature—usually 17–18° C. (62.6–64.4° F.)—favorable for the growth of saprophytic contaminants for a period before testing. The S.P.C. on pasteurized samples held at 7.2° C. for five days gives a good indication of the extent of recontamination, especially with psychrophiles responsible for spoilage during refrigerated storage.

Antibiotics. The presence of antibiotics in milk, either residual from therapy or by deliberate addition, can influence the results of bacteriological examinations (52, 92, 173), in addition to causing trouble in the manufacture of products dependent upon the lactic fermentation and the possible hazard to those individuals acutely sensitive to penicillin. Where the results of bacteriological tests are unexpectedly good, it may be wise to test for the presence of antibiotics.

Unfortunately, there is no rapid test whereby milk containing antibiotics can be detected and rejected. The incorporation of a suitable dye as a tracer has been reported on favorably (64, 149), but the method still lacks official sanction. This would appear to be the most satisfactory solution of this problem; producers would be much less prone to ship milk containing residual antibiotics if they knew that detection was simple. However, this would not preclude the possibility of the deliberate addition of antibiotics as preservatives.

A number of different tests have been reported for the detection of antibiotics in milk. All of these are based upon interference with bacterial growth and activity. One of the simplest is a starter activity test (146), patterned after that introduced by Horrall and Elliker (73); here, the extent of acid development when inoculated with a lactic starter and incubated for several hours is compared with that of a control. Care must be taken, however, to exclude the action of naturally occurring inhibitory substances. Greater sensitivity can be obtained by using *Streptococcus thermophilus* in place of the common starter streptococci (17). Another form of test utilizes a redox indicator to reflect interference with bacterial growth when incubated at a suitable temperature; triphenyl tetrazolium chloride is the indicator commonly recommended (120), although methylene blue (58, 143) and resazurin (115) have been used. The disk assay method has also been studied extensively. In its standard form (2), it is most useful for detecting the presence of penicillin; the test organism, *Bacillus subtilis*, is less sensitive to other antibiotics (88). An interesting modification of this method has recently been described (145), wherein the agar layer is flushed with resazurin; the completed test takes considerably less time than the standard disk assay method.

Hydrolytic rancidity. With the spread of bulk handling of milk there has been heightened interest in the occurrence of hydrolytic rancidity. A useful method for estimating the degree of its development has been developed by Minnesota workers (162).

ESTHETIC CONSIDERATIONS

Sediment. In addition to the microbiological tests already discussed, certain tests are commonly conducted which are concerned with esthetic considerations. Tests for sediment in milk (2) are usually applied to incoming raw milks. Dirt

has no place in milk; hence, its presence indicates carelessness in milking or handling. Milk containing in excess of 2.5 mg. per pint as determined by the off-the-bottom type of tester is customarily rejected. Unfortunately, a relatively clean disk may simply represent a good job of filtering on the farm, rather than care in production.

Different types of apparatus for sediment testing may give quite different results. Preliminary studies (80) have shown that the type operated by compressed air may collect appreciably less sediment on the disk than do certain other types.

There is also a tendency to consider that milk containing appreciable sediment is also high in bacteria. This is not necessarily so. Milk may contain appreciable amounts of sediment, yet show a reasonably low count; on the other hand, milk free from sediment may have picked up a heavy load of contamination from unclean equipment surfaces etc., or appreciable growth may have taken place.

Sediment tests are occasionally run on bottled milks. In most well-operated plants, however, the milk is subjected to filtration or clarification there in addition to straining on the farm, so the amount of sediment present is usually extremely small. Sediment tests for churning cream are also made by enforcement agencies; these require special facilities whereby the entire can of cream may be filtered (66). The visual mold test for cream (2, 129) might also be regarded as a form of sediment test. It is a useful additional criterion of churning-cream quality (122).

Milk for cheese-making may contain appreciable amounts of sediment, which is concentrated approximately elevenfold during the making process. The U. S. Food and Drug Administration has taken the lead in attacking this problem. In Canada since 1954, every vat of Cheddar cheese offered for grading by the federal grading service (111,884 in 1957) has been tested for extraneous matter, using essentially the procedure developed by Thibodeau (161). In 1957, federal grade standards (29) were amended to require a satisfactory extraneous matter test to qualify cheese for First Grade. This has greatly improved the picture, but it is suspected that it represents more effective dirt removal at farm and factory rather than cleaner milk production.

Decomposition. Tests for decomposition also may be regarded as concerned with esthetic considerations. Here tests for water-insoluble acids (WIA) and butyric acid (BA) in butter come to mind (71). Lipolysis in churning cream caused by growth of mold or lipolytic bacteria is reflected in higher values for these compounds in butter, and such evidence may be used by enforcement agencies to take legal action.

The souring of milk and cream also may be regarded as a form of decomposition. The titratable acidity test (2, 153) really measures the buffer capacity of the product. It is still widely used in the grading of churning cream; for market milk on this continent it has all but disappeared, but for manufacturing milk it is still used as a basis for rejection. Its great advantage is the speed with which it can be carried out. Care must be used when applying this test to milk

with high solids-not-fat; cases are known where even certified milk with counts under 10,000 per milliliter has been threatened with rejection because the titratable acidity was high (153). The effect of dilution of the milk or cream with water (2) in reducing the titratable acidity is not generally recognized (154).

The alcohol test (153), still used mainly to determine the suitability of milk for condensing, is primarily a reflection of acid development. Milk giving a positive reaction is usually within a few hours of curdling; on the other hand, some milks quite satisfactory for pasteurization may react positively (32).

Lactic acid is the chief product of bacterial growth in most milk samples; a rapid test for its detection and measurement would be most helpful. Based on the method of Ling (102), Clegg and coworkers (133, 158) have described a rejection test which can be completed in 5 min.; a value of 0.03% is recommended as the lowest value of apparent lactic acid at which milk could be rejected. However, even a 5-min. delay on the receiving platform would disrupt operations in many plants. A test of this type might replace the titratable acidity test in the examination of nonfat milk solids (29); at present careful neutralization makes possible the use of substandard raw material without detection.

Mastitis. While mastitis is primarily a problem of animal health, and occasionally is a factor in the transmission of human pathogenic organisms, the inclusion in the milk supply of the secretion from infected udders also may be regarded as an esthetic problem. Regardless of the use to which it is to be put, no milk can be considered to be of satisfactory quality if drawn from diseased udders. Milk is customarily defined, in part, as the product of the uninterrupted milking of one or more healthy cows, yet the average leucocyte count on samples in the 1953 U. S. survey (38) was 680,000 per milliliter. McKenzie (110) has recently reported that where the leucocyte count exceeded 100,000 per milliliter, the solids-not-fat content was correspondingly reduced. Certainly, a count on herd milk in excess of 500,000 per milliliter strongly suggests an appreciable number of infected udders in the herd.

Various procedures have been suggested for the detection of milks carrying excessive numbers of leucocytes. The direct microscopic examination of smears (2), using a low-powered objective, can yield very useful information in the hands of a trained worker. The ability of resazurin to reflect the weak reducing activity of leucocytes (137) can be used to indicate those herds in need of examination; in either the triple reading test (2, 91) or Hempler's pink test (68, 69), rapid, early color change, followed by a lag for an hour or more, should be regarded with suspicion. Tests can be carried out at the farm, also, preferably using Golding's dried vial technique (108).

A simple rapid procedure, which is rapidly gaining in popularity, is the Whiteside test (117, 171). It is effective in showing differences in herd milks, and can be used as a platform test; it can also be used by the fieldman, or by the farmer himself, to check individual cows or quarters. It has considerable educational value, since the farmer can readily observe how milk from normal and from infected animals differs in appearance. Favorable reports have appeared from various countries; one from Sweden (3) states that the Whiteside test was

more valuable than the catalase or the toxin–blood agar tests, and recommends its adoption. The California Mastitis Test (CMT) (142), developed from the Whiteside test, is designed primarily for checking individual quarter samples; it is much more sensitive than the strip-cup test, and is the basis of a promising mastitis control program (72).

The tests mentioned so far are indirect tests, useful for directing attention to certain milk supplies. A search for specific causative organisms also can be made; information from such tests is a valuable guide in treatment of mastitis. Samples should be taken aseptically from individual quarters. The simplest procedure is probably the Hotis test (74), especially good for detecting *Streptococcus agalactiae*. Use of blood agar, either as streak or pour plates, is considered the most reliable method; the type of colony and hemolysis aids in differentiating various common mastitis organisms. The CAMP test (31) is very useful in identifying *S. agalactiae* by a typical hemolytic reaction. Fuller information on various aspects of mastitis, including testing, is given in Plastridge's review (134).

HEALTHFULNESS

Over the years, milk and its products have been involved in the spread of a number of diseases of human as well as of bovine origin (2). In North America and some European countries, marked progress in the elimination of bovine tuberculosis and brucellosis has sharply reduced the hazard to those still consuming raw milk, while the pasteurization of all but a small fraction of the milk supply has reduced the amount of milk-borne disease to insignificant proportions (39, 150).

Nowadays, routine laboratory testing of raw milk supplies in regard to safety is largely confined to the milk ring test (2) as a screening test to detect brucellosis. This simple, inexpensive test is so sensitive that when applied to pooled herd samples a single infected animal in a herd of 40 can be detected (79). Like all biological tests, it has its limitations. False positive tests occasionally occur in milk from noninfected cows, especially fresh cows or those suffering from mastitis; infected nonlactating animals escape detection, whereas milk from a small percentage of infected animals gives a negative reaction. Despite these limitations, the test has been of inestimable value in programs for the elimination of brucellosis (79).

Phosphatase test. Although it was accepted that efficient pasteurization made milk safe, health officials had no assurance that the procedure had been properly carried out. With the development of the phosphatase test by Kay and Graham (95), a routine analytical method first became available that could give fairly substantial assurance. Underpasteurization, or recontamination with as little as 0.1–0.2% of raw milk or cream will result in a positive reaction. It is still possible for small amounts of underpasteurized milk to be mixed with fully pasteurized milk and escape detection, but judging by the greatly diminished number of outbreaks of milk-borne disease (39, 150), the public health hazard is slight.

Various modifications of the original phosphatase test have been developed; two of these, the New York State Department of Health and the Sanders and Sager methods, are accepted here as official methods (2), whereas the Scharer method is only a screening procedure. A modification developed by Aschaffenburg and Mullen (9) is simple and rapid enough for a routine plant test, yet compares in sensitivity with the Kay-Graham test (95). Directions must be followed carefully, to avoid false positive reactions. Flavors and coloring compounds in ice cream which possess phenolic rings may give false positive reactions. False positive reactions also may result from growth of certain species of microorganisms which produce phosphatase (13, 63). Means of differentiating microbial phosphatases from milk phosphates have been described (98). More recently, reactivation of phosphatase in milk or cream processed at higher temperatures (176) has caused some concern to control officials. More emphasis upon keeping samples cold until tested would go a long way toward the solution of this problem, although an intensive study of reactivation is undoubtedly needed.

Cheese made from raw milk, especially Cheddar and its modifications, has been responsible for numerous outbreaks of enteric disease. Regulations usually require that Cheddar cheese sold when less than 60 days old be made from pasteurized milk. The Sanders and Sager method (141) is a modification of the phosphatase test expressly designed to overcome the strong buffering capacity of cheese and the presence of interfering substances. That of Kosikowski (97) is another. Phosphatase tests for cheese are fully discussed in a recent F.A.O. monograph (98).

While the phosphatase test may be applied to freshly churned butter to determine whether or not it was made from pasteurized cream, unsatisfactory results have attended its application to storage butters (95, 128).

Recently, interest has been aroused by food-poisoning outbreaks attributed to the presence of staphylococcus enterotoxin in nonfat milk solids (4, 8) and in Cheddar cheese and its modifications (6, 159, 160). While toxin production in raw milk itself rarely presents a hazard to health (93), due to the repression of growth of staphylococci by other types, some growth may take place both before and during the cheese-making process (107, 157). Cheese with excessive numbers of coagulase-positive staphylococci must, therefore, be regarded with suspicion (160). Counts of staphylococci in cheese are much higher during the warmer months (157); greater emphasis on prompt cooling of night's milk should reduce the hazard here.

Reliance can not be placed entirely upon low plate counts, however. In the manufacture of powder or in subsequent storage, the staphylococci may be killed off or die, but the enterotoxin remain. This was the case in the Puerto Rico outbreaks (8). Faulty plant practice, rather than excessive growth of staphylococci in milk before it reaches the plant, is more likely to be responsible for the presence of toxin in the powder (4).

GENERAL OBSERVATIONS

There is an unfortunate tendency for many people to forget that bacteriological determinations on milk or milk products are far from being precise

measurements. Making fine distinctions between samples on the basis of small differences in plate counts, for example, is entirely unwarranted, and is expressly discouraged in *Standard Methods* (2). It should further be emphasized that conclusions as to the quality of a milk supply should be drawn only after examining a series of samples, not on the basis of a single sample. This is recognized in the Standard Milk Ordinance (168), where either the logarithmic average of the last four samples is taken or else one high count out of four is not penalized.

Because milks may show appreciable differences in bacteriological content from day to day, it is desirable that testing be carried out as frequently as possible. The less expensive dye reduction tests have an advantage here; they also indicate substandard supplies with a minimum of delay, so that the fieldman can check at once for the source of the trouble.

It should be emphasized once again that the hygienic quality of milk embraces a number of factors. No one method can furnish all the information required in quality control. In addition to estimating the total bacterial population, tests for thermoduric organisms and for excessive leucocyte counts are essential to the control of raw milk supplies; for pasteurized milk the coliform test, or plating after refrigerated storage for several days, is more valuable than the S.P.C. in indicating the care taken to minimize recontamination.

Probably because America has been in the van of progress in respect to milk hygiene, there has been a tendency to overlook testing methods developed elsewhere. This attitude appears to be changing. It seems probable that such developments as the Aschaffenburg and Mullen (9) modification of the phosphatase test, the use of penicillin in media for coliform testing (104, 147), the roll-tube procedure (5), and the coliform test on pasteurized products after previous incubation (16, 18, 125) could be used here to advantage.

The widespread adoption of farm bulk tanks makes it necessary to reappraise standards and methods of testing developed for milk in cans. Some means of encouraging the growth of contaminants prior to testing appears necessary, if bacteriological analysis is to give a reliable indication of the care taken in producing and handling milk. Such a procedure, applied to all milks regardless of the method of handling, appears less open to objection than that of setting up two different standards for acceptable milk (36). Furthermore, the adoption of preincubation would make possible a marked improvement in many milk supplies without altering currently accepted standards for milk handled in cans. In effect, it would mean acceptance of the principle on which control of raw milk has long been based in Britain—that testing freshly taken samples, particularly if milks have been well-cooled, may yield misleading results.

ACKNOWLEDGMENTS

The helpful criticisms of Dr. J. C. Olson of the University of Minnesota, and of Dr. A. H. Robertson of the State Department of Agriculture, Albany, New York, are gratefully acknowledged.

REFERENCES

(1) American Association of Medical Milk Commissions, Inc. *Methods and Standards for the Production of Certified Milk.* New York, New York. 1958.

(2) American Public Health Assoc. *Standard Methods for the Examination of Dairy Products.* 10th ed. New York, New York. 1953.

(3) Anderson, F. Studies of the Applicability of the Whiteside Test on Milk Samples. *Nord. Vet. Med.*, **9**: 191. 1957 (In *Dairy Sci. Abstrs.*, **19**: 665. 1957.)

(4) Anderson, P. H. R., and Stone, D. M. Staphylococcal Food Poisoning Associated with Spray-Dried Milk. *J. Hyg.* (Cambridge), **53**: 387. 1955.

(5) Anonymous. Astell Roll Tube Equipment. *Lab. Practice*, **6**: 635. 1957.

(6) Anonymous. Staphylococcus Food Poisoning from Cheese. *Morbidity and Mortality Repts.*, U.S.P.H.S. September 26, 1958.

(7) Archambault, J. Coliform Densities in Pasteurized Dairy Products. *J. Milk and Food Technol.*, **17**: 342. 1954.

(8) Armijo, R., Henderson, D. A., Timothee, R., and Robinson, H. B. Food Poisoning Outbreaks Associated with Spray-Dried Milks—An Epidemiological Study. *Am. J. Public Health*, **47**: 1093. 1957.

(9) Aschaffenburg, R., and Mullen, J. E. C. A Rapid and Simple Phosphatase Test for Milk. *J. Dairy Research*, **16**: 58. 1949.

(10) Atherton, H. F., Doan, F. J., and Watrous, G. H., Jr. Changes in Bacterial Population and Characteristics of Bottled Market Milk During Refrigerated Holding. Pennsylvania Agr. Expt. Sta., *Bull.* 575. 1954.

(11) Babel, F. J., Collins, E. B., Olson, J. C., Peters, I. I., Watrous, G. H., and Speck, M. L. The Standard Plate Count of Milk as Affected by the Temperature of Incubation. *J. Dairy Sci.*, **38**: 499. 1955.

(12) Barber, F. W., and Fram, H. The Problem of False Coliform Counts on Fruit Ice Cream. *J. Milk and Food Technol.*, **18**: 88. 1955.

(13) Barber, F. W., and Frazier, W. C. The Development of a Positive Phosphatase Test in Refrigerated, Pasteurized Cream. *J. Dairy Sci.*, **26**: 343. 1943.

(14) Barkworth, H., Davis, J. G., Egdell, J. W., Rowlands, A., and Watson, D. W. A Comparison of Rapid Platform Tests for the Bacteriological Quality of Milk. *Proc. Soc. Agr. Bacteriologists*, **25**. 1942.

(15) Barnum, H. J. Laboratory Pasteurization Test to Detect Thermodurics. *Am. Milk Rev. and Milk Plant Monthly*, **21** (2): 110. 1959.

(16) Berger, G., and Anderson, L. Methods for Testing the Keeping Quality of Milk with Special Reference to After-Infection. *Proc. 12th Intern. Dairy Congr.*, **3**: 366. 1949.

(17) Berridge, N. J. Testing Penicillin in Milk. *Dairy Ind.*, **18**: 586. 1953.

(18) Bertelsen, E., Mattsson, N., and Dufeu, J. The Applicability of Bacteriological Tests in Determining the Keeping Quality of Market Milk and Cream. *Proc. 14th Intern. Dairy Congr.*, **3**: 316. 1956.

(19) Bird, E. R., Egdell, J. W., and Thomas, S. B. The Bacteriological Assessment of Milk Production Methods. *Proc. 14th Intern. Dairy Congr.*, **3**: 3. 1956.

(20) Black, L. A. Current Investigations of Laboratory Methods Used in Milk and Food Utensil Sanitation. *J. Milk and Food Technol.*, **11**: 5. 1948.

(21) Black, L. A. Personal communication. 1957.

(22) Bouska, F. W. The Significance of Yeasts and Oidia in Pasteurized Butter. Univ. of Wisconsin Studies in Science, No. 2. 1921.

(23) Boyd, J. C., Smith, C. K., and Trout, G. M. The Effect of the Incubation Time and Temperature on the Determination of Psychrophilic Bacteria in Milk by the Agar Plate Method. *J. Milk and Food Technol.*, **17**: 365. 1954.

(24) Breed, R. S. The Determination of the Number of Bacteria in Milk by Direct Microscopic Examination. *Centr. Bakteriol. II Abt.*, **30**: 337. 1911.

(25) Breed, R. S. Simplification of Laboratory Control Procedures for Market Milk. *Am. J. Public Health*, **33**: 386. 1943.

(26) BROITMAN, S., MALLMANN, W. L., AND TROUT, G. M. A Simple Test for Detecting Keeping Quality for Milk. *J. Milk and Food Technol.*, **21**: 280. 1958.

(27) BUCHBINDER, L., AND ALFF, E. C. Studies on Coliform Organisms in Dairy Products. *J. Milk and Food Technol.*, **10**: 137. 1947.

(28) BURRI, R. The Quantitative Milk Culture. A Simple Means for the Bacteriological Examination of Milk. *Proc. World's Dairy Congr.*, 690. 1928.

(29) CANADA DEPARTMENT OF AGRICULTURE. Canada Dairy Products Act. 1951, as amended March 21, 1957.

(30) CHALMERS, C. H. The Bulk Cooling, Storage and Transport of Milk. Vol. 14, No. 1. Health Bull., Dept. of Health (Scotland). 1956.

(31) CHRISTIE, R., ATKINS, N. E., AND MUNCH-PETERSEN, E. A Note on a Lytic Phenomenon Shown by Group B Streptococci. *Australian J. Exptl. Biol. and Med. Sci.*, **22**: 197. 1944.

(32) CLEGG, L. F. L. Rejection Tests for Raw Milk. A Review of the Literature. *Dairy Sci. Abstrs.*, **14**: 731. 1952.

(33) CLEGG, L. F. L., EGDELL, J. W., THOMAS, S. B., MCKENZIE, D. A., AND CUTHBERT, W. A. Thermoduric Organisms in Milk. Part II. Preliminary Studies on Testing Techniques. *Proc. Soc. Appl. Bacteriol.*, **13**: 132. 1950.

(34) CLEGG, L. F. L., THOMAS, S. B., AND COX, C. P. A Comparison of Roll-Tube and Petri Dish Colony Counts on Raw Milk. *Proc. Soc. Appl. Bacteriol.*, **14**: 171. 1951.

(35) CLEGG, L. F. L., WOHLGEMUTH, J. L., CROSSLEY, E. L., AND ANDREWS, L. E. The Influence of Sterility of Farm Dairy Equipment on the Bacteriological Quality of Raw and Laboratory Pasteurized Milk. *Proc. Soc. Appl. Bacteriol.*, **2**: 43. 1949.

(36) CORASH, P. The Production and Handling of Quality Milk. *J. Milk and Food Technol.*, **19**: 277. 1956.

(37) CUTHBERT, W. A., THOMAS, S. B., EGDELL, J. W., NICHOLS, A. A., AND COX, C. P. Thermoduric Organisms in Milk. Part IV. Colony Counts of Thermoduric Organisms Obtained with Different Incubation Times at 30°. *J. Appl. Bacteriol.*, **17**: 182. 1954.

(38) DAHLBERG, A. C., ADAMS, H. S., AND HELD, M. E. Sanitary Milk Control and Its Relation to the Sanitary, Nutritive and Other Qualities of Milk. *Natl. Research Council (U.S.), Publ.* 250. 1953.

(39) DAUER, C. C. 1957 Summary of Disease Outbreaks. *J. Milk and Food Technol.*, **21**: 338. 1958.

(40) DAVIS, J. G., AND NEWLAND, L. G. The Use of the Resazurin Comparator in Artificial Light. *Dairy Ind.*, **8**: 555. 1943.

(41) DAY, E. A., AND DOAN, F. J. A Test for the Keeping Quality of Pasteurized Milk. *J. Milk and Food Technol.*, **19**: 63. 1956.

(42) DEPT. OF HEALTH (Scotland). Scottish Milk Testing Scheme. *Memo.* No. 33. 1955.

(43) DERBY, H. A., AND HAMMER B. W. Bacteriology of Butter. IV. Bacteriological Studies on Surface Taint Butter. Iowa Agr. Expt. Sta., *Research Bull.* 145. 1931.

(44) DONNELLY, C. B., BLACK, L. A., HARRIS, E. K., AND LEWIS, K. H. An Evaluation of Simplified Methods for Determining Viable Counts of Raw Milk. Paper presented at 59th Gen. Meeting, Soc. Am. Bacteriol., St. Louis, Mo. 1959.

(44a) EDDISON, R. T., KEMPTHORNE, Q., HOSKING, Z., BARKWORTH, H., COX, C. P., AND ROWLANDS, A. Keeping Quality and Raw-Milk Grading. II. The Effect of Storage on the Results of Keeping Quality and Dye Tests. *J. Dairy Research*, **18**: 43. 1951.

(45) EDDLEMAN, T. L., AND BABEL, F. J. Phosphatase Reactivation in Dairy Products. *J. Milk and Food Technol.*, **21**: 126. 1958.

(46) EGDELL, J. W., THOMAS, S. B., CLEGG, L. F. L., AND CUTHBERT, W. A. Thermoduric Organisms in Milk. Part III. Provisional Standard Technique for the Laboratory Pasteurization Test for Milk and Rinses and Swabs of Dairy Equipment, with Suggestions for Interpretation of Results. *Proc. Soc. Appl. Bacteriol.*, **13**: 132. 1950.

(47) EGDELL, J. W., THOMAS, S. B., CUTHBERT, W. A., JONES, G. E., AND HOSKING, Z. D. Thermoduric Organisms in Milk. Part V. A Simplified Testing Technique. *J. Appl. Bacteriol.*, **19**: 256. 1956.

(48) Ehrlich, R. Membrane Filter Method for Determination of Coliforms in Pasteurized and Certified Milk. *J. Milk and Food Technol.*, **16**: 6. 1953.

(49) Erdman, I. E., and Thornton, H. R. Psychrophilic Bacteria in Edmonton Milk and Cream. I. Numbers. *Canadian J. Technol.*, **29**: 232. 1951.

(50) Fifield, C. W., Hoff, J. E., and Proctor, B. E. The Millipore Filter for Enumerating Coliform Organisms in Milk. *J. Dairy Sci.*, **40**: 588. 1957.

(51) Fischer, J. B., and Johns, C. K. A Comparison of Various Methods for Detecting Thermoduric Bacteria in Milk. *J. Milk Technol.*, **5**: 269. 1942.

(52) Foley, E. J., and Byrne, J. V. Penicillin as an Adjunct to the Preservation of Quality of Raw and Pasteurized Milk. *J. Milk and Food Technol.*, **13**: 170. 1950.

(53) Food and Drugs (England). The Milk (Special Designation) (Pasteurized and Sterilized Milk) Regulations, 1949. H.M. Stationery Office, London.

(54) Forest, H. L., and Small, E. The Direct Microscopic Clump Count as a Measure of Quality of Non-Fat Dry Milk. *Proc. 15th Intern. Dairy Congr.*, **3**: 1883. 1959.

(55) Forg, F. J. "Bacto-Strip": A Simple Bacteriological Routine Test Method. *Lab. Practice*, **5**: 439. 1956.

(56) Frost, W. D. Comparison of a Rapid Method of Counting Bacteria in Milk with the Standard Plate Method. *J. Infectious Diseases*, **19**: 273. 1916.

(57) Funder, S., and Johannessen, S. Can the Membrane Filter Method Be Used to Advantage in Bacteriological Routine Control Work? *Nord. Mejeritidsskr.*, **23**: 41. 1957. (In *Dairy Sci. Abstrs.*, **19**: 576. 1957.)

(58) Galesloot, T. E. The Detection of Penicillin in Milk and Dairy Products. *Netherlands Milk and Dairy J.*, **9**: 158. 1955.

(59) Galesloot, T. E. The Laboratory Examination of Milk in Relation to the Cleanliness of the Methods of Production on the Farm. *Intern. Dairy Fed.*, Doc. No. 4. 1956.

(60) Galesloot, T. E. Het Tellen van Coli-Aerogenes Bacterien in Melk mit Bactostrips van Bacto Strip A.G. te Zurich. *Netherlands Milk and Dairy J.*, **10**: 166. 1956.

(61) Golding, N. S. The Resazurin Reductase Test Using Prepared Sterile Dry Vials. *J. Dairy Sci.*, **30**: 521. 1947.

(62) Griffiths, D. G., Druce, R. G., and Thomas, S. B. Advisory Microbiological Standards and Tolerances for Raw Milk. *J. Appl. Bacteriol.*, **20**: 243. 1957.

(63) Hammer, B. W., and Olson, H. C. Phosphatase Production in Dairy Products by Microorganisms. *J. Milk Technol.*, **4**: 83. 1941.

(64) Hargrove, R. E., Plowman, R. D., and Wright, W. W. Use of Markers in Veterinary Preparations for the Detection of Antibiotics in Milk. *J. Dairy Sci.*, **42**: 202. 1959.

(65) Harris, E. K., Thomas, R. C., and Black, L. A. A Statistical Analysis of Reduction Times in Relation to Plate Counts. *J. Milk and Food Technol.*, **19**: 243. 1956.

(66) Harris, K. L., and Warden, L. L. Portable Equipment for Whole-Can Testing of Cream for Extraneous Matter. *Milk Prod. J.*, **45** (3): 32. 1954.

(67) Heinemann, B., and Rohr, M. R. A Bottle Agar Method for Bacterial Estimates. *J. Milk and Food Technol.* **16**: 133. 1953.

(68) Hempler, P. Resazurin as Indicator in Reduction Tests of Milk. Dissertation, A/S Carl Fr. Mortensen, Copenhagen. 1950.

(69) Hempler, P. Grading of Milk Supplies by Means of Resazurin Tests. *Proc. 13th Intern. Dairy Congr.*, **2**: 255. 1953.

(70) Hempler, P. Psychrophilic Bacteria in Pasteurized Milk. *Nord. Vet. Med.*, **7**: 705. 1955. (In Danish.) (See *Dairy Sci. Abstrs.*, **18**: 244. 1956.)

(71) Hillig, F. Determination of Water-Insoluble Fatty Acids in Cream and Butter. *J. Assoc. Offic. Agr. Chemists*, **30**: 575. 1947.

(72) Holt, D. Mastitis Can Be Licked Now. *Hoard's Dairyman*, **104**: 231. March 10, 1959.

(73) Horrall, B. E., and Elliker, P. R. An Activity Test for Cheddar and Cottage Cheese Starters. *J. Dairy Sci.*, **30**: 523. 1947.

(74) Hotis, R. P., and Miller, W. T. A Simple Method for Detecting Mastitis Streptococci in Milk. USDA, *Circ.* 400. 1936.

(75) HOWARD, I. A., AND FISCHER, B. M. Colony Count Determinations by the Roll-Tube Method in Commercial Practice. *Dairy Inds.*, **15**: 918. 1950.
(76) HUMPHRISS, E. L. E. Ice Cream. *Food Mfr.*, **34**: 62-64. 1959.
(77) INTERNATIONAL DAIRY FEDERATION. Rept. Intern. Comm. Standardization of Methods of Analysis of Milk and Milk Products, 1958.
(78) JAMIESON, M. C., AND CHEN, H. K. A Swab-Slant Test for Equipment Sanitation and the Correlation with a Standard Method. *J. Milk Technol.*, **8**: 134. 1945.
(79) JANNEY, G. C., BERMAN, D. T., AND ERDMAN, A. A. The Relative Efficiency of the Milk Ring Test and the Area Blood Tests for Bovine Brucellosis. *J. Am. Vet. Med. Assoc.*, **133**: 586. 1958.
(80) JEWSON, J. F. Personal communication. June 1, 1959.
(81) JEZESKI, J. J., OLSON, J. C., JR., AND LAWTON, W. C. Comparative Studies of Bacteriological Methods for the Grading of Raw Milk. *Proc. Milk Ind. Found.* (Lab. Sect.), 44, 1955.
(82) JOHNS, C. K. A Rapid Method for Determining the Mold and Yeast Count of Butter. *Sci. Agr.*, **8**: 353. 1928.
(83) JOHNS, C. K. Factors Influencing the Reduction of Methylene Blue in Milk. *Sci. Agr.*, **19**: 435. 1939.
(84) JOHNS, C. K. Place of the Methylene Blue and Resazurin Reduction Tests in a Milk Control Program. *Am. J. Public Health*, **29**: 239. 1939.
(85) JOHNS, C. K. Dye Concentration in Resazurin Tablets. *Am. J. Public Health*, **34**: 955. 1944.
(86) JOHNS, C. K. Unpublished results. 1957-58.
(87) JOHNS, C. K. Detecting Recontamination of Pasteurized Products. *Canadian Dairy and Ice Cream J.*, **38**: 34. 1959.
(88) JOHNS, C. K., AND BERZINS, I. Observations on the Determination of Antibiotics in Milk. *J. Milk and Food Technol.*, **19**: 14. 1955.
(89) JOHNS, C. K., AND BERZINS, I. The Bacto-Strip Technique for Making Counts of Bacteria, Molds and Yeasts. *J. Milk and Food Technol.*, **20**: 345. 1957.
(90) JOHNS, C. K., AND BERZINS, I. The Value of Preliminary Incubation in Bacteriological Tests for Milk. *Proc. 15th Intern. Dairy Congr.*, **3**: 1293. 1959.
(91) JOHNS, C. K., AND HOWSON, R. K. A Modified Resazurin Test for the More Accurate Estimation of Milk Quality. *J. Milk Technol.*, **3**: 320. 1940.
(92) JOHNS, C. K., AND KATZNELSON, H. Penicillin and Dye Reduction Tests for Milk Quality. *J. Milk and Food Technol.*, **12**: 133. 1949.
(93) JONES, A. C., KING, G. J. C., FENNELL, H., AND STONE, D. The Growth of *Staphylococcus aureus* in Milk with Special Reference to Food Poisoning. Monthly Bull., Med. Research Council (Britain), **16**: 109. 1957.
(94) KAY, H. D., CUTTELL, J. R., HALL, H. S., MATTICK, A. T. R., AND ROWLANDS, A. Milk Pasteurization. Planning, Plant, Operation and Control. F.A.O. Agr. Studies, No. 23, Rome, Italy. 1953.
(95) KAY, H. D., AND GRAHAM, W. R., JR. Phosphorus Compounds in Milk. VI. The Effect of Heat on Milk Phosphatase. A Simple Method for Distinguishing Raw from Pasteurized Milk, Raw from Pasteurized Cream and Butter Made from Raw Cream from That Made from Pasteurized Cream. *J. Dairy Research*, **5**: 63. 1933.
(96) KNAYSI, G. On the Use of Basic Dyes for the Demonstration of the Hydrolysis of Fat. *J. Bacteriol.*, **42**: 587. 1941.
(97) KOSIKOWSKI, F. V. A Simple Universal Dairy Products Phosphatase Test. *Science*, **110**: 480. 1949.
(98) KOSIKOWSKI, F. V., AND MOCQUOT, G. Advances in Cheese Technology. F.A.O. Agr. Studies, No. 38: Rome. 1958.
(99) LAWTON, W. C. Personal communication. March 20, 1959.
(100) LEVINE, B. S. Effect of Formaldehyde on the Direct Microscopic Count of Raw Milk. Public Health Rept., **65**: 931. 1950.

(101) Levowitz, D. An Enquiry into the Usefulness of the Standard Methods Direct Microscopic Count. *J. Milk and Food Technol.*, **20**: 288. 1957.

(102) Ling, E. R. The Determination of Lactic Acid in Milk. *J. Sci. Food Agr.*, **2**: 279. 1951.

(103) Malcolm, J. F., and Leitch, R. H. The Value of the Methylene Blue Reduction Test in the Grading of Milk. *Scottish J. Agr.*, **19**: 321. 1936.

(104) Malling Olsen, E. Use of Penicillin in Liquid and Solid Coliform Media. *Proc. 13th Intern. Dairy Congr.*, **3**: 1302. 1953.

(105) Mallmann, W. L., and Broitman, S. A Surface Plating Technic for Determining Bacterial Population of Milk. *Am. J. Public Health*, **46**: 1018. 1956.

(106) Mallmann, W. L., Bryan, C. S., and Fox, W. K. A New Microscopic Procedure for the Detecting and Locating of the Source of Thermoduric Organisms in Milk. *J. Milk Technol.*, **4**: 195. 1941.

(107) Mattick, A. T. R., Neave, F. K., and Chapman, H. R. *Staphylococcus aureus* in Cheddar Cheese. *Proc. 15th Intern. Dairy Congr.*, **3**: 1914. 1959.

(108) McBride, C. A., and Golding, N. S. A Study of Resazurin Reduction in Freshly Drawn Mastitis-Like Milk. *J. Milk and Food Technol.*, **14**: 27. 1951.

(109) McCrady, M. H., and Langevin, E. M. The Coli-Aerogenes Determination in Pasteurization Control. *J. Dairy Sci.*, **15**: 321. 1932.

(110) McKenzie, D. A., Booker, E. M. K., and Moore, W. Observations on the Cell Count and S.N.F. Content of Cows' Milk. *J. Dairy Research*, **25**: 52. 1958.

(111) Mickle, F. L., and Borman, E. K. The Connecticut Three-Point Laboratory Program as an Aid to Control of Pasteurized Milk. *J. Milk Technol.*, **6**: 231. 1943.

(112) Ministry of Agriculture (England). Guide to the Conduct of Clean Milk Competitions. Misc. Publ. No. 43. 3rd ed. 1928.

(113) Ministry of Health (England). Bacteriological Tests for Graded Milk. *Memo.* 139 Foods, H.M. Stationery Office, London. 1937.

(114) Moir, G. M., and Russell, R. R. Bacteriological Testing of Butter. New and Simplified Routine Methods. *J. Dairy Research*, **10**: 310. 1939.

(115) Moldavan, A. Decelage des Antibiotiques dans le Lait. *Quebec Laitier*, **10** (3): 24. 1951.

(116) Morgan, M. E., Anderson, E. O., and Plastridge, W. N. Mastitis and the Plate Count of Milk. II. The Influence of *Streptococcus agalactiae* Mastitis upon Standard Plate Count of Milk. *J. Milk Technol.*, **5**: 67. 1942.

(117) Murphy, J. M., and Hanson, J. J. A Modified Whiteside Test for the Detection of Chronic Bovine Mastitis. *Cornell Vet.*, **31**: 47. 1941.

(118) Mustakallio, K. K., Ahos, E. O., and Autio, E. O. Letter. *Science*, **122**, No. 3177. November 18, 1955.

(119) Myers, R. P., and Pence, J. A. A Simplified Procedure for the Laboratory Examination of Raw Milk Supplies. *J. Milk Technol.*, **4**: 18. 1941.

(120) Neal, C. E., and Calbert, H. E. The Use of Tetrazolium Salts as Indicators for Lactic Starter Inhibitory Substances. *Proc. 14th Intern. Dairy Congr.*, **2**: 359. 1956.

(121) Nelson, F. E., and Baker, M. P. The Influence of Time and Temperature of Plate Incubation upon the Bacterial Counts of Market Milk and Related Products, Particularly After Holding Under Refrigeration. *J. Milk and Food Technol.*, **17**: 95. 1954.

(122) Nelson, F. E., Martin, W. H., Morrison, R. W., and Caulfield, W. J. An Evaluation of the Visual Mold Test for Cream. *J. Dairy Sci.*, **26**: 375. 1943.

(123) New Hampshire State Dept. of Health. Laws and Regulations Relating to Dairy Products. Concord, N.H. 1955.

(124) Olsen, H. C., and Bonner, M. D. Methods for Analysis of Cottage Cheese. Oklahoma Agr. Expt. Sta., *Tech. Bull.* T-71. 1957.

(125) Olsen, S. J. Determination of the Keeping Qualities of Milk. *Proc. 14th Intern. Dairy Congr.*, **3** (2): 339. 1956.

(126) Olson, J. C., Jr., Willoughby, D. S., Thomas, E. L., and Morris, H. A. The Keeping Quality of Pasteurized Milk as Influenced by the Growth of Psychrophilic Bacteria and the Addition of Aureomycin. *J. Milk and Food Technol.*, **16**: 213. 1953.

(127) Overby, A. J. Bedmmelse of produktionsmaelkens hygiejniskbakteriologiske Kvalitat. (English summary.) *Maelkeritidende*, **68**: 1. 1955.

(128) Parfitt, E. H., Brown, W. H., and Shadwick, G. W., Jr. Application of the Phosphatase Test to Butter. *Am. J. Public Health*, **30**: 240. 1940.

(129) Parsons, C. H. A Visual Mold Test for Cream. *Natl. Butter and Cheese J.*, **32**: (3) 12, 13, 56-59. 1941.

(130) Pederson, C. S., and Yale, M. W. Effect of Temperature of Incubation upon Agar Plate Count of Milk. *Am. J. Public Health*, **24**: 477. 1934.

(131) Pelczar, M. J., Jr., and Brown, J. H. Synthetic Culture Media for Reference Use in Dairy Bacteriology. *J. Milk and Food Technol.*, **14**: 90. 1951.

(132) Pesch, K. L., and Simmert, V. Untersuchungen über den Frischeitzustand der Milch. Bestimmung der Wasserstoffionen-Konzentration und der Reductionzeit. *Milchwirtsch. Forsch.*, **8**: 551. 1929.

(133) Pickering, A., and Clegg, L. F. L. A Modified Procedure for the Determination of Developed Lactic Acid as a Rejection Test for Raw Milk with a Consideration of the Relevance of the Test. *Dairy Ind.*, **23**: 325-328. 1958.

(134) Plastridge, W. N. Bovine Mastitis. A Review. *J. Dairy Sci.*, **41**: 1141. 1958.

(135) Prescott, S. C. The Need for Uniform Methods in the Sanitary Examination of Milk. U.S. Public Health Papers and Rept., **31**: (2): 246. 1905.

(136) Provan, A. L., and Rowlands, A. Dye Reduction Tests for Heat Treated Milk. *Dairy Ind.*, **8**: 693. 1943.

(137) Ramsdell, G. A., Johnson, W. T., Jr., and Evans, F. R. Investigation of Resazurin as an Indicator of the Sanitary Condition of Milk. *J. Dairy Sci.*, **18**: 705. 1935.

(138) Regulation (1949). The Milk (Special Designation) (Raw Milk) Regulations No. 1590, 1949. H.M. Stationery Office, London, England. 1949.

(139) Report Coli-Aerogenes Sub-Committee. The Nomenclature of Coli-Aerogenes Bacteria. *J. Appl. Bacteriol.*, **19**: 108. 1956.

(140) Report, Committee on Applied Laboratory Methods, I.A.M.F.S. *J. Milk and Food Technol.*, **21**: 107. 1958.

(141) Sanders, G. P., and Sager, O. S. Development of a Phosphatase Test Applicable to Cheddar Cheese. *J. Assoc. Offic. Agr. Chemists*, **28**: 656. 1945.

(142) Schalm, O. W., and Noorlander, D. O. Experiments and Observations Leading to Development of the California Mastitis Test. *J. Am. Vet. Med. Assoc.*, **130**: 199. 1957.

(143) Schipper, I. A., and Petersen, W. E. Assay of Aureomycin in Milk by Use of Methylene Blue. *Vet. Med.*, **46**: 222. 1951.

(144) Sedgewick, W. T., and Batchelder, J. L. A Bacteriological Examination of the Boston Milk Supply. *Boston Med. and Surg. J.*, **126**: 25. 1892.

(145) Shahani, K. M., and Badami, M. C. A Resazurin Disk Assay Method for Detecting Antibiotics and Natural Starter Inhibitory Activity in Milk. *J. Dairy Sci.*, **41**: 1510. 1958.

(146) Silverman, G. J., and Kosikowski, F. V. Systematic Testing of Inhibitory Substances in Milk. *J. Milk and Food Technol.*, **15**: 120. 1952.

(147) Simonart, P., and Lambert, R. Suggestions pour le Controle Microbiologique des Laits. *Proc. 13th Intern. Dairy Congr.*, **2**: 317. 1953.

(148) Smillie, D. M. Coliform Contamination of Farm Milks. *Dairy Ind.*, **18**: 580. 1953.

(149) Smitasiri, T., Kosikowski, F. V., Guthrie, R. S., and Fincher, M. G. Dyes as Markers for Antibiotic-Contaminated Milks. *J. Milk and Food Technol.*, **21**: 255. 1958.

(150) Smith, J. Pathogenic Organisms in Dairy Products. *Dairy Sci. Abstrs.*, **18**: 451. 1956.

(151) Smythe, V. R. Keeping-Quality Test for Pasteurized Milk in Warm Climates. *Proc. 15th Intern. Dairy Congr.*, **3**: 1312. 1959.

(152) Smythe, V. R. Significance of Thermoduric Bacteria in Milk Supplies. *Proc. 15th Intern. Dairy Congr.*, **3**: 1898. 1959.

(153) SOMMER, H. H. *Market Milk and Related Products.* 3rd ed. Published by the author, Madison, Wisconsin. 1952.

(154) SOMMER, H. H., AND MENOS, J. The Effect of Dilution on the Titratable Acidity of Cows' Milk. *J. Dairy Sci.*, **14**: 136. 1931.

(155) STIRLING, A. C., STEVENS, M. K., AND LAWLEY, D. N. Colony Counts on Strips of Agar in Tubes. *J. Gen. Microbiol.*, **4**: 339. 1950.

(156) STOLTZ, E. I., AND HANKINSON, D. J. The Effects of Antibiotics on the Bacterial Plate Counts of Normal Raw Milk. *J. Milk and Food Technol.*, **16**: 157. 1953.

(157) TAKAHASHI, I., AND JOHNS, C. K. *Staphylococcus aureus* in Cheddar Cheese. *J. Dairy Sci.*, **42**: 1032. 1959.

(158) TAYLOR, P. B., AND CLEGG, L. F. L. A Rejection Test for Raw Milk. *J. Dairy Research*, **25**: 32. 1958.

(159) THATCHER, F. S. Extraneous Matter and Bacteria of Public Health Significance in Cheese. *Canadian J. Publ. Health*, **47**: 234. 1956.

(160) THATCHER, F. S. Personal communication. May 6, 1959.

(161) THIBODEAU, R. Extraneous Matter in Canadian Cheddar Cheese. *Canadian Dairy and Ice Cream J.*, **26** (10): 31. 1947.

(162) THOMAS, E. L., NIELSEN, A. J., AND OLSON, J. C., JR. Hydrolytic Rancidity in Milk. A Simplified Method for Estimating the Extent of Its Development. *Am. Milk Rev.*, **17** (1): 50. 1955.

(163) THOMAS, S. B. Thermophilic Bacteria in Raw and Pasteurized Milk. *Welsh J. Agr.*, **13**: 295. 1937.

(164) THOMAS, S. B. Coli-Aerogenes Bacteria in the Bovine Udder. *Dairy Sci. Abstrs.*, **17**: 2. 1955.

(165) THOMAS, S. B. Coli-Aerogenes Bacteria in Raw Milk. *J. Appl. Bacteriol.*, **18**: 331. 1955.

(166) THOMAS, S. B., AND TUDOR, J. A. The Comparative Values of the Colony Count on Milk Agar, the Coliform Test and the Methylene Blue Reductase Test for the Grading of Raw Milk. *Welsh J. Agr.*, **13**: 308. 1937.

(167) TIEDEMAN, W. D., AND HOHL, N. J. Improved Laboratory Control of Pasteurized Milk. *Am. J. Public Health*, **28**: 629. 1938.

(168) U.S. PUBLIC HEALTH SERVICE. Milk Ordinance and Code: *Public Health Bull.* 220. 1953.

(169) WATROUS, G. H., JR., DOAN, F. J., AND JOSEPHSON, D. V. Some Bacteriological Studies on Refrigerated Milk and Cream. Pennsylvania State Agr. Expt. Sta., *Bull.* 551. 1952.

(170) WEBB, A. H., AND HUMPHRIES, F. A Comparison of the Kay-Graham and the Aschaffenburg-Mullen Phosphatase Tests for Pasteurized Milk. *Dairy Ind.*, **19**: 305. 1954.

(171) WHITESIDE, W. H. Observations on a New Test for the Presence of Mastitis in Milk. *Canadian Public Health J.*, **30**: 44. 1939.

(172) WILDMAN, J. D. Development of Methods for the Estimation of Mold in Cream and Butter. *J. Assoc. Offic. Agr. Chemists*, **20**: 93. 1937.

(173) WILKOWSKE, H. H., AND KRIENKE, W. A. Influence of Penicillin in Milk on Total and Coliform Bacteria Plate Counts. *J. Milk and Food Technol.*, **14**: 92. 1951.

(174) WILSSENS, A., AND DE VLEESCHAUWER, A. The Coliform Test at 30° C. for Pasteurized Milk. *Mededel. Landbouwhogeschool en Opzoekingsstas. Staat. Gent.*, 1, **21**: 125. 1956. (In *Dairy Sci. Abstrs.*, **18**: 1053. 1956.)

(175) WILSON, G. S. The Bacteriological Grading of Milk. Med. Research Council, London, *Special Rept.* 206. 1935.

(176) WRIGHT, R. C. The Influence of Reactivation of Phosphatase upon the Assessment of the Heat Treatment of Milk and Cream. *Proc. 14th Intern. Dairy Congr.*, **3** (2): 717. 1956.

CHARACTERISTICS OF HIGH-MELTING TRIGLYCERIDE FRACTIONS FROM THE FAT-GLOBULE MEMBRANE AND BUTTER OIL OF BOVINE MILK[1]

M. P. THOMPSON,[2] J. R. BRUNNER, AND C. M. STINE
Department of Dairy—Food Technology Program, Michigan Agricultural Experiment Station, East Lansing

SUMMARY

A high-melting triglyceride fraction (HMGF) was isolated from an alcohol-ether extract of the fat-globule membrane and from an ethanol extract of butter oil. The HMGF was characterized by its iodine value (4.8-5.0), saponification equivalent (200-202), and melting range (50.0-52.5° C.), and by its ultraviolet (220-340 mμ), near-infrared (1.6-3.1 μ), and infrared (2-14 μ) spectra. The fatty acid composition of the fraction was determined by converting the glyceride to its constituent fatty acid methyl esters which were resolved by gas chromatography as follows: C_{10} — 0.1, C_{12} — 0.9, C_{14} — 11.0, C_{16} — 59.6, C_{18} — 16.5, $C_{18}^{1=}$ — 5.6, $C_{18}^{2=}$ — 0.4, $C_{18}^{3=}$ — 0.2, and unassigned components—5.8 mole %, respectively. Characteristics of the high-melting glyceride fractions obtained from the membrane and from butter oil were similar. The HMGF constitutes the triglyceride content of the membrane.

In 1933, Palmer and Wiese (6) reported the isolation of a high-melting triglyceride from the fat-globule membrane prepared from washed cream. Later, Jenness and Palmer (4) published specific chemical properties of the ethanol-insoluble fraction extracted at 24–25° C. from buttermilk, butter serum, and butter oil. A recent note by Patton and Keeney (7) gave characteristics of a glyceride isolated from an acetone extract of milk fat. This paper presents the results of a study conducted independently from but concurrently with the recent work of Patton and Keeney. The results presented here are based on a high-melting triglyceride fraction (HMGF) isolated from the fat-globule membrane and a similar fraction obtained from butter oil as ethanol-insoluble fractions at 22° C.

METHODS

The iodine values, saponification equivalents, and melting-range values were determined according to standard procedures (1).

Ultraviolet and near-infrared spectra of the HMG Fractions and other selected materials were obtained with a Beckman Model DK-2 ratio-recording spectrophotometer.

A Beckman Model IR-5 double-beam infrared spectrophotometer was employed to study the spectral range from 2 to 14 μ. Samples were carried in spectrophotometric-grade carbon tetrachloride.

Received for publication January 2, 1959.

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal article No. 2365.

[2] This article is part of a dissertation to be presented to the Faculty of the Graduate School of Michigan State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The HMG Fractions and butter oil were prepared for gas chromatographic analyses by methanolysis in Skellysolve A, according to the method described by Smith and Jack (9). Excess solvent was removed in vacuo. The resulting fatty-acid methyl-ester mixtures were resolved on a Burrell gas chromatographic unit. Identification of the unknown chromatographic peaks was achieved by comparing their elution characteristics with those from similarly prepared chromatograms of a known fatty acid mixture, and by employing the known mixture as an internal standard. The chromatographic column was packed with 18.6 g. of 20% (w/w) Reoplex 400 on 60–80 mesh Celite 545 and was operated at a temperature of 224° C. The detector was maintained at 238° C. while the preheater was operated at 263° C. Helium was employed as the carrier gas, with an outflow rate of approximately 50 cc/38.9 sec. at C_{18} for all chromatographic runs.

EXPERIMENTAL PROCEDURE

Preparation of the high-melting triglyceride. The glyceride fraction was obtained from the concentrated fat-globule membrane prepared from washed cream (3). The membrane material was washed several times with peroxide-free ethyl ether at approximately 20° C., to remove fat which was not associated with the membrane complex. Lipids complexed within the membrane were extracted with a 35% ethanol in ethyl ether mixture for 15 min. at −10° C., followed by filtration and two washings with cold ether to remove the excess ethanol. Five additional extractions with ether at 35° C. were employed to remove the last traces of lipids from the membrane proteins. These alcohol and ether extracts were combined and evaporated to dryness in vacuo at 40° C. The residual lipids were taken up in five volumes of acetone. The mixture was allowed to stand for 30 min., after which the phospholipid precipitate was filtered off and the clear filtrate evaporated to dryness in vacuo. At this point, it should be realized that acetone does not remove quantitatively the heterogeneous phospholipid mixtures (5). The remaining phospholipids were not recovered in the HMG Fractions.

The crude glyceride was dissolved in 600 ml. of absolute ethanol at 35° C. and allowed to stand overnight at 21–23° C. to allow crystallization of the HMGF. The copious white precipitate was separated from the yellow supernatant by vacuum filtration. This crystallized fraction was purified further by four crystallizations from absolute ethanol at 21–23° C. The purified glyceride fraction was freed of ethanol in vacuo and stored under nitrogen until analyzed. Similarly, 100 g. of anhydrous butteroil was extracted with 1,500 ml. of ethanol at 35° C. and the HMGF allowed to crystallize at 21–23° C. The precipitate was treated similarly to the membrane HMGF and represented the HMGF of butter oil.

Following each crystallization of the membrane HMGF, a sample of the ethanol supernatant was analyzed in the ultraviolet region, to determine the extent of removal of ethanol-soluble materials which, if present, would serve to mask the true characteristics of the HMGF. These spectra are shown in Figure 1. The characteristics of the spectra differ markedly as the number of crystallization steps is increased. Following the third extraction, the spectrum

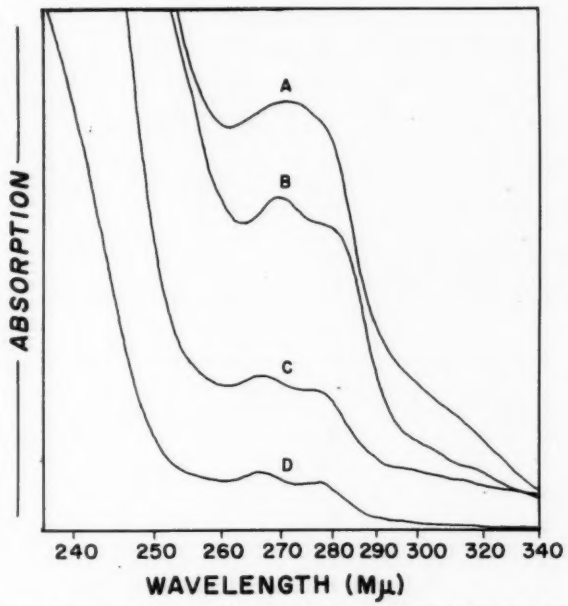


FIG. 1. Ultraviolet spectra of ethanol extracts of the high-melting triglyceride. Curve A, first extract, diluted 1: 2, 0.1 cm. path. Curve B, second extract, diluted 1: 1, 0.1 cm. path. Curve C, third extract, undiluted, 0.1 cm. path. Curve D, fourth extract, undiluted, 1.0 cm. path.

of the ethanol supernatant began to resemble that of the HMGF (Figure 2, B). The first and second extractions, which were yellow in color, showed spectra quite dissimilar to those of the subsequent extractions. Obviously, this was due to the presence of absorbing substances such the sterols, beta carotene, or phospholipids. These data indicate, then, that four extractions with absolute ethanol were required to free the membrane HMGF of its major impurities. The ethanol extractions were combined, evaporated to dryness in vacuo, and retained for further study.

Characterization of the high-melting triglycerides. The iodine value, saponification equivalent, and melting range for the HMG Fractions of this study were compared with similar values for the glyceride fractions reported by Jenness and Palmer (4) and by Patton and Keeney (7), Table 1. A difference exists between the reported iodine values of the two HMG Fractions reported here and the HMGF obtained by acetone fractionation from milk fat (7). This difference might be attributed to the method of preparation; that is, ethanol extraction as opposed to acetone extraction.

The ultraviolet spectra of the HMG Fractions from the fat-membrane and butter oil are compared in Figure 2. These glycerides show identical spectral characteristics with five absorption maxima at 315, 301, 279, 267, and 256 mμ, respectively. Smith and Jack (9), reporting on the unsaturated fatty acids of

TABLE 1

Properties of the HMGF isolated from the fat-globule membrane and from milk fat

Analysis	From the fat membrane (this study)	From milk fat (this study)	From the fat membrane (4)	From milk fat (7)
Iodine value	5.0	4.8–4.9	5.0–7.1	2.0
Saponification equivalent	201–202	200–202	198.8–204	....[c]
Melting range	50–51.5° C.	50–52.5° C.	52–53° C.	....[c]
Refractive index at 60° C.	1.4455	1.4453	.....	....[c]
Yield	44.48[a]	4.88%[b]		

[a] Percentage of bound lipid in membrane.
[b] Percentage of original butter oil.
[c] Reported by Keeney and Patton (7) to be similar to values reported by Jenness and Palmer (4).

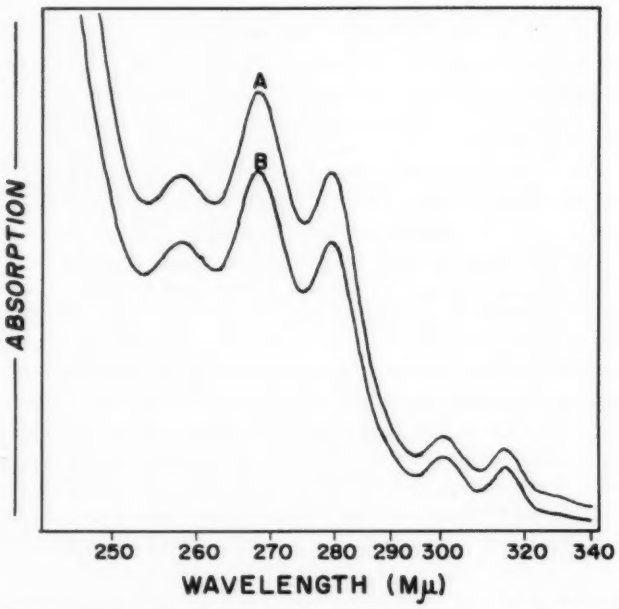


FIG. 2. Ultraviolet absorption spectra of the high-melting glyceride fractions obtained from (A) butter oil and (B) the fat-globule membrane.

milk fat, present almost identical spectral characteristics, showing absorption peaks at approximately 313, 300, 280, 268, and 233 mμ, but not at 256 mμ. These spectral qualities characterize the presence of unsaturation in the glycerides. However, the data can not be interpreted to determine the type of conjugation, since alkali isomerization was not employed.

In recent years, the near-infrared region of the spectrum (2.7–3.0 μ) has proven useful for the study of functional groups and has been especially useful in the identification of hydroperoxides (8). Figure 3 shows the near-infrared

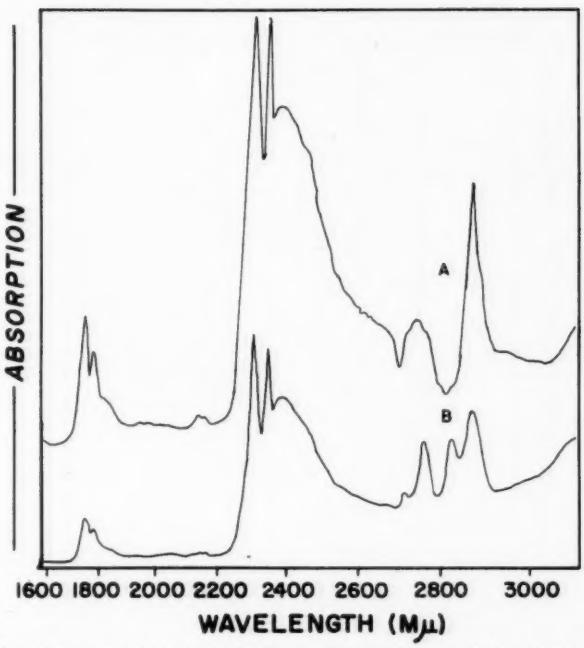


FIG. 3. Near-infrared (photoelectric) absorption spectra of (A) tristearin and (B) the high-melting triglyceride in CCl_4. Concentrations approximate 5%.

spectra for the membrane HMGF, and tristearin. An examination of these spectra reveals that there was little similarity between these compounds in the region of 2.7–3.0 μ. Obviously, this dissimilarity arises from the nature of the molecule, i.e., saturation or unsaturation.

The infrared spectra (2–14 μ) of the HMG Fractions and tristearin are shown in Figure 4. Although this spectral region is most useful in assigning functional groups, one must be cautioned against over-simplification when interpretations are based on these data alone. In the case of lipids, for example, the spectra of the fatty acids correspond closely to related spectra of triglycerides. One would expect this correlation because of the similarities in the functional groups of the related compounds. The esters, on the other hand, differ in their spectral characteristics, depending on the type of ester (i.e., methyl or ethyl). The spectra of tristearin and of the HMG Fractions were quite similar and corresponded to those published by Patton and Keeney (7).

Gas chromatograms of the methyl esters of the fatty acids of butter oil and the membrane HMGF from the same milk, and a mixture of known fatty acids (C_{10} to C_{18}, and $C_{18}{}^{=1}$ to $C_{18}{}^{3=}$) are shown in Figure 5. These chromatograms were analyzed and compared with similar data reported by Patton and Keeney (7), Table 2. The membrane HMGF contained approximately 9 *M* % more C_{16} and 9 *M* % less C_{18} saturated acids, and 2.0 *M* % more C_{18} unsaturated acids

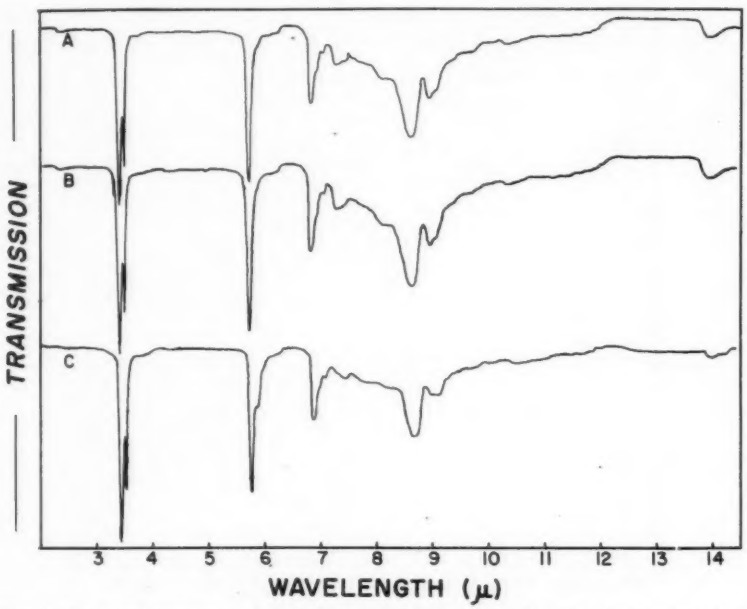


FIG. 4. Infrared spectra of (A) HMGF from butter oil, (B) HMGF from the fat-globule membrane, and (C) tristearin. Concentration was approximately 5%.

TABLE 2

A comparison of the fatty acid composition of the fat-globule membrane HMFG, butter oil from the same preparation, and a HMFG isolated from milk fat

Fatty acid	HMGF from the fat membrane[a]	Butter oil	HMGF from milk fat (7)	
	(*M* %)			
			A–1	B–1
C_{10}	0.1	3.9	0.3	0.7
C_{12}	0.9	4.3	1.4	1.6
C_{14}	11.0	12.6	12.1	13.6
C_{16}	59.6	25.5	50.5	51.6
C_{18} saturated	16.5	11.3	25.5	30.4[c]
C_{18} unsaturated	6.1	28.3	4.1[b]	.....
$C_{18}^{1=}$	5.6	25.8		
$C_{18}^{2=}$	0.4	.3		
$C_{18}^{3=}$	0.2	2.2		
Unassigned peak areas	5.8	10.5	5.9	.....

[a] Identical gas chromatograms were obtained for both the membrane and butter oil HMG Fractions.
[b] Total C_{18} unsaturated.
[c] Includes C_{18} unsaturated.

than the glyceride reported by them. Specifically, the C_{18} unsaturated acids have been designated as $C_{18}^{1=}$—5.6 *M* %, $C_{18}^{2=}$—0.4 *M* %, and $C_{18}^{3=}$—0.2 *M* %. No analysis was made for $C_{18}^{4=}$. Unassigned peaks accounted for 5.8 *M* % of the total mixture. The data for the butter oil sample were presented merely for the purpose of establishing the nature of original milk fat.

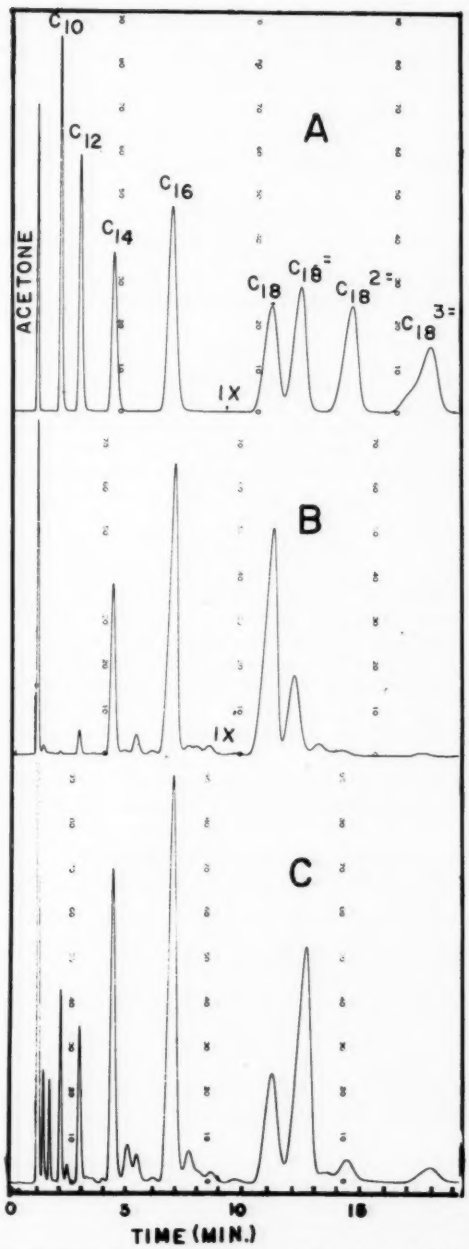


FIG. 5. Gas chromatograms of the fatty acid methyl esters of (A) a known mixture of fatty acids, (B) the fat-globule membrane HMGF, and (C) butter oil. 1x denotes time of a twofold increase in detector sensitivity.

Interestingly, the chromatogram of the esterified ethanol-soluble fraction of the membrane showed only traces of fatty acids, indicating a complete recovery of the HMGF from the ethanol crystallization step, as well as suggesting that the entire membrane triglyceride is HMGF. A classification of the unsaponifiable materials (beta carotene, sterols, etc.), constituting the major portion of this fraction, is currently in progress in this laboratory.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. Edward M. Priest, M.D., and his associates in the Division of Cardiology at the Henry Ford Hospital, Detroit, for performing the gas chromatographic analyses of the methylated fatty acid samples reported in this paper.

REFERENCES

(1) Association of Official Agricultural Chemists. *Official Methods of Analysis.* 8th ed. George Banta Publ. Co., Menasha, Wisconsin. 1955.

(2) Deuel, H. J., Jr. *The Lipids, Their Chemistry and Biochemistry.* Vol. I. *Chemistry.* Interscience Publ., Inc., New York. 1951.

(3) Herald, C. T., and Brunner, J. R. The Fat-Globule Membrane of Normal Cow's Milk. I. The Isolation and Characteristics of Two Membrane-Protein Fractions. *J. Dairy Sci.*, **40**: 948. 1957.

(4) Jenness, R., and Palmer, L. S. Substances Adsorbed on the Fat Globules in Cream and Their Relation to Churning. VI. Relation of the High-Melting Triglyceride Fraction to Butterfat and the "Membrane." *J. Dairy Sci.*, **28**: 653. 1945.

(5) Lucas, C. C., Peterson, Jean M., and Ridont, Jessie H. Solubility of Tissue Phosphatides in Acetone. *Arch. Biochem. and Biophys.*, **78**: 546. 1958.

(6) Palmer, L. S., and Wiese, H. F. Substances Adsorbed on the Fat Globule in Cream and Their Relation to Churning. II. The Isolation and Identification of Adsorbed Substances. *J. Dairy Sci.*, **16**: 41. 1933.

(7) Patton, R., and Keeney, P. The High-Melting Glyceride Fraction from Milk Fat. *J. Dairy Sci.*, **41**: 1288. 1958.

(8) Slover, H. T., and Dungan, L. R., Jr. Application of Near Infrared Spectrophotometry to the Study of Autoxidation Products of Fats. *J. Am. Oil Chemists' Soc.*, **35**: 350. 1958.

(9) Smith, L. M., and Jack, E. L. The Unsaturated Fatty Acids of Milk Fat. II. Conjugated and Nonconjugated Constituents. *J. Dairy Sci.*, **37**: 390. 1954.

COMPARISON OF NUCLEIC ACIDS AND OTHER PHOSPHORUS COMPOUNDS OF TERRAMYCIN-SENSITIVE AND -RESISTANT STRAINS OF *STREPTOCOCCUS LACTIS*[1]

K. M. SHAHANI[2]
Department of Dairy Technology, The Ohio State University, Columbus

SUMMARY

Terramycin-sensitive and terramycin-resistant strains of *Streptococcus lactis* 10A were inoculated in an enriched broth and incubated at 30° C. After 6, 12, and 24 hr. of incubation, determinations were made for total cellular matter, acid-soluble phosphorus, lipid phosphorus, nucleic acid phosphorus, protein phosphorus, and ribonucleic and deoxyribonucleic acids.

The sensitive strain consistently produced more cellular matter than did the resistant strain. The concentrations of the various phosphorus fractions fluctuated during incubation. In general, acid-soluble phosphorus was present in the highest concentrations in cells of both the strains, followed by protein phosphorus, lipid phosphorus, and nucleic acid phosphorus, in that order. At all times, cells of the sensitive strain contained higher concentrations of various phosphorus fractions, except for the acid-soluble fraction, which was present in higher concentration in the resistant cells after 6 and 12 hr. of incubation, and except for the lipid phosphorus content, which was higher in the resistant cells after a 12-hr. incubation period. The ribonucleic acid contents of both cultures were three to four times higher than their deoxyribonucleic acid contents. The total nucleic acid content of the sensitive cells during the earlier stages of incubation was appreciably greater than that of the resistant cells, indicating that the sensitive culture synthesized the nucleic acids more rapidly. However, the total nucleic acid content of the sensitive cells varied widely in different trials. The total nucleic acid content in both the strains decreased after 12 and 24 hr. of incubation, but the extent of decrease in the sensitive strain was more rapid than in the resistant strain, with the result that after 24 hr. of incubation, the total nucleic acid content of both the strains was approximately the same.

With the thought that antibiotic-resistant cultures may lend themselves to use in the manufacture of cultured products from antibiotic-milk, four strains of *Streptococcus lactis* were made resistant to 100 p.p.m. of terramycin (1, 15). Physiological studies on terramycin-sensitive and terramycin-resistant strains of *S. lactis* 10A, reported elsewhere (1, 14, 15, 16), have revealed considerable physiological differences between the two strains with respect to their cell number multiplication, acid production, utilization of phosphate esters and radioactive phosphorus, and metabolism of certain carbohydrates. In general, it was observed that the resistant strain metabolized at a reduced rate compared to the sensitive strain.

Nucleic acids combined with protein constitute an essential part of all animal and plant cell-nuclei which play an important part in the physiological functioning of the cells. Because of the physiological differences between the sensitive

Received for publication October 16, 1958.

[1] Article No. 12: 58 of the Department of Dairy Technology, The Ohio State University. Supported by a grant from the U. S. Public Health Service (National Institutes of Health) and by the Ohio Dairy Products Research Fund.

[2] Present address: Department of Dairy Husbandry, University of Nebraska, Lincoln.

and resistant strains of *S. lactis* 10A observed in previous studies, it was felt that differences might exist in their nucleic acid and nucleoprotein metabolism. These studies were conducted to investigate whether there existed any differences between the nucleic acid, nucleoprotein, and other phosphorus-containing fractions of the terramycin-sensitive and -resistant strains of *S. lactis* 10A.

EXPERIMENTAL PROCEDURE

The terramycin-sensitive and -resistant strains of *S. lactis* 10A were grown in an enriched broth (pH 6.8) consisting of 0.5 g. yeast extract, 0.5 g. lactose, 0.5 g. dibasic potassium phosphate, 0.25 g. neopeptone, and 0.25 g. peptonized milk per 1,000 ml. Freshly prepared medium was inoculated with 5% of a 12-hr. broth culture and incubated at 30° C. At the end of 6, 12, and 24 hr. of incubation, the cells were collected by centrifugation, washed two or three times with sterile water, and suspended in 10 ml. of sterile water. The total dry cellular material produced at the end of each time-interval was determined on a 2- to 5-ml. aliquot of the suspension by means of the Mojonnier technique (9). Another aliquot was used for the determination of various phosphorus fractions and nucleic acids by employing the step-wise fractionation procedure of Schneider (11, 12). The cell-suspension was mixed with a small quantity of sand and ground for 10 min. with a pestle and mortar to facilitate extraction of various phosphorus fractions. The phosphorus fractions determined were acid-soluble phosphorus, lipid phosphorus, nucleic acid phosphorus, and phosphoprotein phosphorus. The acid-soluble phosphorus fraction was extracted with 10% cold trichloroacetic acid and separated from the residue by centrifugation. The lipid phosphorus fraction was extracted from the residue with alcohol and hot alcohol : ether treatment. After the removal of the second fraction, the nucleic acid phosphorus fraction was extracted by boiling the residue with 5% trichloroacetic acid. Finally, the protein-attached phosphorus was determined after alkali hydrolysis of the residue. In all of the fractions, phosphorus was determined by the standard Fiske and Subbarow Method (7). Ribonucleic acid (RNA) was determined by the orcinol-pentose colorimetric method of Brown (4), as described by Schneider (12). Since orcinol reacts with both ribonucleic acid and deoxyribonucleic acid (12), a correction was applied for the presence of deoxyribonucleic acid. Deoxyribonucleic acid (DNA) was determined by the diphenylamine reaction of Dische and Schwarz (6), as modified by Schneider (12). The procedures for the nucleic acids have been described fully by Volkin and Cohn (17). Commercially available ribonucleic acid and deoxyribonucleic acid [3] were used for preparation of the standard curves for nucleic acids. All results presented in this study are expressed on a dry weight basis.

RESULTS AND DISCUSSION

The dry cellular matter produced by the sensitive and resistant strains of *S. lactis* 10A is illustrated by Figure 1. Dry cellular weight at zero-hour incu-

[3] Obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio.

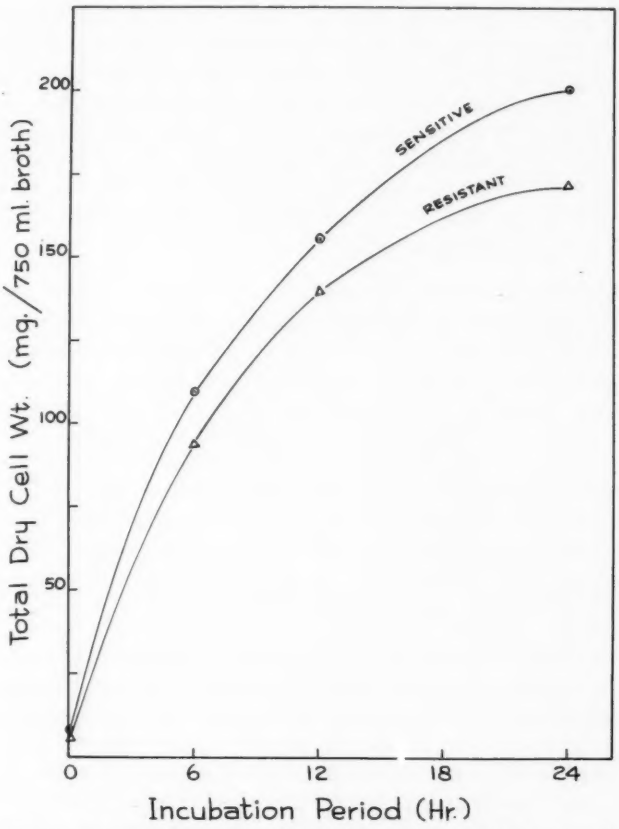


FIG. 1. Total dry cellular matter produced by terramycin-sensitive and -resistant *S. lactis* 10A. These data represent average values of three to four trials conducted with each culture at each time of incubation.

bation represents the estimated values calculated from the amount of the inoculum. The resistant strain produced 10 to 14% less cellular material than did the sensitive strain. In a previous study (15), the rate of multiplication of the two strains was found to be the same, as determined by the viable cell total count. In the present work, the growth was determined by the total dry cellular matter produced by the organisms at various time-intervals of incubation. The variations in the two studies might be due either (a) to the production of longer streptococcal chains by the sensitive strain which may be more resistant to being shaken apart during dilution, (b) to the production of smaller-sized cells by the resistant strain, or (c) to the fact that the sensitive strain multiplied faster and approached the decline phase earlier than the other strain and, thereby, the dead cells of the sensitive strain contributed to the higher cellular weight.

The data for the various phosphorus fractions are presented (Table 1). At the end of 6 and 12 hr. of incubation, the resistant cells usually contained a higher concentration of acid-soluble phosphorus but a lower concentration of lipid, nucleic acid, and protein-bound phosphorus than the sensitive cells. However, at the end of 24 hr. of incubation, all of the phosphorus fractions were present in lower concentrations in the resistant cells than in the sensitive ones. In general, the acid-soluble phosphorus was present in the highest concentration in cells of both strains, followed in order by the protein phosphorus, lipid phosphorus, and nucleic acid phosphorus.

TABLE 1

Phosphorus content of the terramycin-sensitive and -resistant *Streptococcus lactis* 10A[a]

Phosphorus fraction	*S. lactis* 10A	Phosphorus content mg/g		
		6 hr.	12 hr.	24 hr.
Acid soluble-P	Sensitive	3.66	4.54	5.58
	Resistant	4.22	6.20	3.11
Lipid-P	Sensitive	2.15	1.72	2.12
	Resistant	1.69	1.80	1.94
Nucleic Acid-P	Sensitive	1.56	1.28	1.47
	Resistant	1.25	0.95	1.19
Protein-P	Sensitive	2.92	3.48	2.08
	Resistant	2.57	3.11	1.91

[a] These data represent the average results of from three to five trials.

Results of nucleic acid analyses are presented in Table 2. The average nucleic acid content in the resistant cells was lower than that in sensitive organisms, at all incubation intervals. Wide variations existed in the nucleic acids content of the two strains in different trials. At the end of 6 hr., the resistant cells contained 31.9 to 40.3 mg/g (average 35.4 mg/g) of the nucleic acids, compared to 38.3 to 56.1 mg/g (average 50.0 mg/g) by the sensitive cells. At 12 and 24 hr. of incubation, the cells of both the strains contained a lower concentration of

TABLE 2

Nucleic acid content of the terramycin-sensitive and -resistant *Streptococcus lactis* 10A at the end of various incubation periods

Trial No.	Compound	Sensitive			Resistant		
		6 hr.	12 hr.	24 hr.	6 hr.	12 hr.	24 hr.
		(mg/g)					
1	Ribonucleic Acid	50.9	18.0	25.5	29.7	16.8	21.6
2		28.6	26.4	20.6	21.1	22.8	19.3
3		45.4	18.8	34.7	33.1	27.2	30.7
4		.....	33.9	.....	.....	24.1	.....
Average		41.6	24.3	26.9	27.9	22.7	23.9
1	Deoxyribonucleic Acid	5.2	6.8	9.8	4.5	2.9	12.6
2		9.7	10.7	7.0	10.8	7.7	7.9
3		10.3	8.5	9.4	7.2	6.1	8.8
4		.....	10.8	.....	.....	5.8	.....
Average		8.4	9.2	8.7	7.5	5.6	9.8
Average total nucleic acid		50.0	33.5	35.6	35.4	28.3	33.7

these acids than they did at 6 hr. In both strains and at all times, ribonucleic acid was more predominant than deoxyribonucleic acid, and constituted 72 to 83% of the total nucleic acid content in the sensitive cells and 71 to 80% of the total nucleic acid content in the resistant cells.

The finding of higher concentrations of ribonucleic acid in the young cells than in the older cells seems to corroborate the report of Morse and Carter (10), who observed that *Escherichia coli* synthesized a large amount of ribonucleic acid during the lag period. Also, Belozersky (2) has shown that the nucleic acid of bacteria, yeast, and mold varied between 3 and 29%, depending upon their age.

Comparison was made of two methods of determining nucleic acid–phosphorus—one involving the actual determination of phosphorus and the other involving calculation of phosphorus from the nucleic acids found (Table 3). It was observed that nucleic acid–phosphorus actually determined (Column 5) was always lower than the nucleic acid–phosphorus calculated from the nucleic acids found (Column 4). Schneider (12) observed that although all of the phosphorus in the trichloroacetic acid extract of animal tissues could be accounted for by nucleic acids, the nitrogen found was always higher than that calculated from the nucleic acids present. He suggested that this could mean either that the nucleic acids present did not conform to the accepted tetranucleotide structure or that some other nitrogenous compound was being extracted by the acid treatment. In the present study, the disagreement between the nucleic acid–phosphorus actually found, and that calculated, may suggest either that in the process of extracting the nucleic acid fraction all of the terminal phosphorus groups can not be extracted or that the phosphorus contents of the nucleic acids in the sensitive and resistant *S. lactis* are less than the accepted phosphorus content of nucleic acids.

Sevag *et al.* (13) have shown that in 16-hr.-old cells of *Streptococcus pyogenes* total nucleic acids (determined on the basis of purine nitrogen) constituted 14.8 to 24.1% of the dry weight of the cells. In the present study, the nucleic acids content (determined on the basis of their sugar content) of the sensitive and resistant strains of *S. lactis* was found to vary from 3.3 to 5.0% and from

TABLE 3

Comparison of two methods for determining total nucleic-acid-phosphorus of the terramycin-sensitive and -resistant *Streptococcus lactis* 10A

Culture	Incubation time	Total[a] nucleic acids	Nucleic Acid[b] calculated	Nucleic Acid[c] found
	(hr.)	*(milligrams per gram dry cells)*		
Sensitive	6	50.0	4.85	1.56
Resistant	6	35.4	3.43	1.25
Sensitive	12	33.5	3.25	1.28
Resistant	12	28.3	2.76	0.95
Sensitive	24	35.6	3.44	1.47
Resistant	24	33.7	3.26	1.19

[a] From Table 2. Determined on the basis of their ribose and deoxyribose content.
[b] Theoretically, ribonucleic acid of yeast contains 9.5 to 9.64% phosphorus, and deoxyribonucleic acid of thymus contains 9.89% phosphorus (12, 13).
[c] From Table 1.

2.8 to 3.5%, respectively. This difference between the two studies may be due to physiological differences between *S. lactis* and *S. pyogenes* or to the different methods used in obtaining the nucleic acid values. Nevertheless, there was agreement between the ratios of deoxyribonucleic acid to total nucleic acid found in *S. lactis* and in *S. pyogenes*. In the present work, deoxyribonucleic acid constituted 17 to 28% of the total nucleic acid content of *S. lactis*, compared to 10 to 30% in *S. pyogenes* (13). Also, Cohen (5) has reported that normal *E. coli* cells synthesize three times as much ribonucleic as deoxyribonucleic acid.

Previously, it was observed that the resistant strain utilized P^{32} at a slower rate than the normal strain (15). Also, Miura *et al.* (8) have reported that the inhibitory effect of aureomycin and terramycin on staphylococci is related to their inhibition of the transfer of high-energy phosphate bonds. On the basis of the present results, it would appear that during the development of antibiotic-resistance, the phosphorylation reactions of the parent *S. lactis* organisms were affected, and that the emerging mutant strain was physiologically incapable of utilizing as much phosphorus for nucleic acid synthesis as its parent strain.

Boivin (3) has reported that nucleic acids, combined with proteins, constitute an essential part of all animal and plant cell-nuclei and play an important part in the physiological functioning of the cells. The differences in the nucleic acids metabolism of the sensitive and resistant strains as found in this study might be associated with the physiological differences observed earlier (1, 14, 15) between the two strains.

ACKNOWLEDGMENTS

Appreciation is expressed to Dr. I. A. Gould for his interest and counsel in this study. Also, grateful acknowledgment is made to Dr. R. C. Ottke, Chas. Pfizer and Co., Inc., for supplying the terramycin hydrochloride used in this study.

REFERENCES

(1) Angelotti, R. Studies on the Physiological Activities of Terramycin-Resistant and -Susceptible Cultures of Cheese Ripening Strains of *Streptococcus lactis*. Ph.D. thesis, Ohio State University, 1955.

(2) Belozersky, A. N. On the Nucleoproteins and Polynucleotides of Certain Bacteria. *Proc. Cold Spring Harbor Symposia Quant. Biol.*, **12**: 1. 1947.

(3) Boivin, A. Directed Mutation in Colon Bacilli by an Inducing Principle of Deoxyribonucleic Nature: Its Meaning for the General Biochemistry of Heredity. *Proc. Cold Spring Harbor Symposia Quant. Biol.*, **12**: 7. 1947.

(4) Brown, A. H. Determination of Pentose in the Presence of Large Quantities of Glucose. *Arch. Biochem.*, **11**: 269. 1946.

(5) Cohen, S. S. The Synthesis of Bacterial Viruses. I. The Synthesis of Nucleic Acid and Protein in *Escherichia coli* B Infected with T_{2r} + Bacteriophage. *J. Biol. Chem.*, **174**: 281. 1948.

(6) Dische, Z., and Schwarz, K. Mikromethods zur Bestimung Verschiedener Pentosen nebeneinender bei Gegenwart Von Hexosen. *Mikrochim. Acta*, **2**: 13. 1937.

(7) Fiske, C. H., and Subbarow, Y. The Colorimetric Determination of Phosphorus. *J. Biol. Chem.*, **66**: 375. 1925.

(8) Miura, Y., Nakamura, Y., Yoshizawa, Y., and Matsdaira, H. Comparative Studies on the Phosphorus Metabolism of Staphylococci in the Presence of Chlortetracycline and Oxytetracycline. *Antibiotics & Chemotherapy*, **3**: 822. 1953.

(9) MOJONNIER MILK TESTER INSTRUCTION MANUAL. *Bull.* No. 101. Mojonnier Bros. Co., Chicago. 1925.

(10) MORSE, M. L., AND CARTER, C. E. The Synthesis of Nucleic Acids in Cultures of *Escherichia coli*, Strains B and B/R. *J. Bacteriol.*, **58**: 317. 1949.

(11) SCHNEIDER, W. C. Nucleic Acids and the Cancer Problem. Ph.D. thesis, University of Wisconsin. 1945.

(12) SCHNEIDER, W. C. Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Desoxypentose Nucleic Acid and Pentose Nucleic Acid. *J. Biol. Chem.*, **161**: 293. 1945.

(13) SEVAG, M. G., SMOLENS, J., AND LACKMAN, D. B. The Nucleic Acid Content and Distribution in *Streptococcus pyogenes*. *J. Biol. Chem.*, **134**: 523. 1940.

(14) SHAHANI, K. M. Carbohydrate and Pyruvate Metabolism of Oxytetracycline-Sensitive and Oxytetracycline-Resistant Organisms. Antibiotics Annual 1956-57, p. 523. Medical Encyclopedia, Inc. 1957.

(15) SHAHANI, K. M., AND HARPER, W. J. Differences in the Acid Production and Phosphorus Metabolism of Oxytetracycline Sensitive and Resistant Organisms. *Appl. Microbiol.*, **6**: 9. 1958.

(16) SOBOL, F. S. A Comparative Study of the Amino Acids Requirements of Terramycin-Resistant and Terramycin-Sensitive Strains of *Streptococcus lactis*. M.S. thesis, Ohio State University, 1957.

(17) VOLKIN, E., AND COHN, W. E. Estimation of Nucleic Acids. *Methods Biochem. Anal.*, **1**: 287. 1954.

USE OF THE SELECTION INDEX METHOD TO ACCOUNT FOR THE NUMBER OF DAUGHTERS AND RECORDS IN PROGENY-TESTING A DAIRY BULL

S. R. SEARLE[1]
New Zealand Dairy Board, Wellington

SUMMARY

Factors have been developed for taking account of the number of daughters and records in the progeny-test of a dairy bull. They are obtained by evaluating the regression of half the sire's additive genetic value on a function which compares the bull's daughter level to an expected daughter level. An extract from the table of the factors is given.

The selection index method of ranking animals according to predicted values of their genetic merit has been proposed on many occasions [Hazel (2) and Lush (4)]. Although it is a method which is theoretically simple, its application sometimes involves cumbersome algebraic expressions. In such cases, practical usage demands simplification, usually through extensive approximations. This paper outlines the process of such a series of approximations used in New Zealand when ranking dairy bulls, and shows how an unwieldy formula was reduced to a table of working values.

The progeny test of dairy bulls in New Zealand since 1950 has been based on comparing a bull's daughter average with the daughter average that would be expected in a herd of the production level in which the bull has been used. Most of the 3,000 proofs published annually in the Sire Survey (7), and earlier, are one-herd natural service proofs and, as such, are based on widely differing numbers of daughters and lactations. This paper shows how the proof has been amended to take account of these numbers, and discusses similar amendments for proofs of bulls used in artificial breeding. A table is given showing the factors used for making these amendments.

Difference from expectancy. Prior to the changes which this paper reports, bulls have been ranked by an expression known as the difference from expectancy. In computing this, the butterfat average for a bull's daughters is calculated each year, after age-correcting the records of the 2- and 3-yr.-olds. The average of the mature cows (ages 4–9 yr.) in the same herd, excluding any of the bull's daughters, is also calculated. From the latter, an expected daughter average is obtained from a regression line which has been estimated on many occasions for all proven bulls.

$$E\,(\text{daughter average}) = 76 + .75\,(\text{mature cow average}),$$

E being expectation over sires. The difference from expectancy for a bull is

Received for publication March 18, 1959.

[1] Present address: Department of Animal Husbandry, Cornell University, Ithaca, New York.

then the difference between his actual daughter average and this expected daughter average, namely

$$D.F.E. = \text{daughter average} - E\,(\text{daughter average}).$$

When a bull has daughters milked in more than 1 yr., the yearly values of this expression are averaged, weighted by the number of daughters milked each year, so that

$$\begin{aligned} D.F.E. &= \bar{d} - 76 - .75\bar{m} \\ &= \sum_i (d_i - .75 n_i \bar{m}_i)/L - 76 \end{aligned} \tag{1}$$

where L is the total number of daughter lactations in the proof, $\Sigma\, d_i$ is the sum over years of year totals for daughters, n_i is the number of daughters in the proof in year i, and $\bar{m}_i$ is the mature cow average in year i.

In publishing the proofs, the numbers of daughters and records in each proof were and are still shown; and the proofs used to be broadly classified as Preliminary, Intermediate, or Final, according to these numbers. This enabled the reader to assess the reliability of the proof for any bull, according to his own judgment of the relative importance of the number of daughters and records. This subjective method of weighing the results has now been replaced by factors which convert the difference from expectancy into an index now published as a rating.

Regression coefficients as factors. The object of sire selection indices is to rank bulls according to their breeding values. The breeding value of a bull includes both additive and nonadditive genetic parts, and by using a suitable mathematical model a prediction of the additive genetic part can be obtained by finding the regression of the bull's additive genetic value on a function of his daughters' records. Suppose this regression is

$$E(S) = \alpha + \beta\,(D.F.E.) \tag{2}$$

where S is the additive genetic value of a bull, $D.F.E.$ is the difference from expectancy, and E is expectation over the population of bulls. For ranking purposes, the α can be omitted, and since the additive genetic contribution of a bull to his daughters is $S/2$, the estimate of this which is suitable for ranking purposes is $\beta(D.F.E.)/2$. This is the estimated additive genetic superiority of the bull's daughters over those of an average bull, out of average dams in both cases.

Thus, $\beta/2$ is the factor used to correct the difference from expectancy into an index, now published as a rating. β would be the same for all bulls if they all had the same pattern of daughter and record numbers, and ranking of $D.F.E.$ alone would then suffice. This, however, is not the case. Most bulls in New Zealand are one-herd sires initially with few daughters and records in their proofs, the numbers increasing after the bull has been used for a year or two. These numbers affect the regression coefficients, and the problem was to develop an expression for β, then simplify it and reduce it to a workable form, in terms of the number of daughters and records.

Mathematical model. The mathematical model used for the record of the k^{th} daughter by the j^{th} sire made in the i^{th} year of the sire's proof was

$$d_{ijk} = \mu + \frac{1}{2}S_j + \frac{1}{2}D_{jk} + G'_{jk} + P_{jk} + Y_{ij} + e_{ijk} \qquad (3)$$

where μ is the mean yield of all daughters;

S_j is the additive genetic value of the j^{th} sire;

D_{jk} is the additive genetic value of the dam of the k^{th} daughter of the j^{th} sire;

G'_{jk} is the additive genetic value of the daughter over and above $\frac{1}{2}(S_j + D_{jk})$, due to Mendelian sampling;

P_{jk} represents nonadditive genetic effects and permanent environmental effects peculiar to the daughter;

Y_{ij} is the effect of the i^{th} year within the herd in which the j^{th} bull was used; and e_{ijk} is the residual error peculiar to this record.

All terms in the model, except μ, were assumed to be random variables with zero means; the variances of S_j and D_{jk} were assumed equal, to σ_g^2, and that of G'_{jk}, was taken as $\sigma_g^2/2$ and the variances of P_{jk}, Y_{ij}, and e_{ijk} were taken as σ_p^2, σ_Y^2, and σ_e^2, respectively. Each set of terms (the S's D's ...) was considered to be a set of uncorrelated variables, each set being independent of the other.

These assumptions mean that the breeding value of a bull is uncorrelated with the breeding value of the cows to which he is mated; they also imply that the genetic variance among the bulls a farmer uses in his herd is the same as that among his cows, and that this is the same for all farmers. With one-herd bulls these assumptions will be untrue to the extent of the consistency with which a farmer can pick bulls as good as or better than the cows in his herd. The assumptions also imply that the effects on production due to years are uncorrelated, which is untrue when there are any carry-over effects from one year to the next; furthermore, it would probably be more correct to treat year effects as fixed rather than random. Despite these and no doubt other weaknesses in the model, it is expedient from a mathematical viewpoint to introduce no further terms.

When a sire's proof extends over more than 1 yr. (as is frequently the case), the required regression term involves correlations that utilize the following parameters:

$$r = \text{repeatability} = (\sigma_g^2 + \sigma_p^2)/\sigma^2$$
$$h = \text{within-herd heritability} = \sigma_g^2/\sigma^2$$
$$t = \text{"seasonability"} = \sigma_Y^2/\sigma^2$$

σ_g^2 is the additive genetic variance within a herd, so that h as here defined is heritability in the "narrow sense"; σ^2 is the variance of a record d_{ijk}. The following table shows the appearance of these parameters in correlations between various pairs of records that can form part of the difference from expectancy expression.

Interpretation of this table is exemplified by considering the terms in the first

TABLE 1

Correlations between all possible pairs of records that can exist in a proof

Year in which record is made	Animals making records									
	Daughters		Mature cows		Daughter Dam		Daughter	Mature cow, not the daughter's dam
	j	j'	k	k'	(jk)	(jk)		
i	$\leftarrow h/4+t \rightarrow$		$\leftarrow t \rightarrow$		$\leftarrow h/2+t \rightarrow$		$\leftarrow t \rightarrow$	
	r	$h/4$	r		r	$h/2$		
i'								

cell; the correlation between the record of daughter j in year i and its record in year i' is r; the correlation between its record in year i and that of its half-sib, j', in the same year is $(h/4 + t)$; and the correlation between half-sib records made in different years i and i', is $h/4$.

Assumptions. The first assumptions applied to the model to obtain the regression were

(i) that one-third of the daughters milked in any year have their dams milked in the same year,

(ii) that no daughters milked in any year of the proof have their dams milked in some other year of the proof, and

(iii) that when a daughter goes out of production it does not return to the herd again.

With these assumptions and parameters, it can be shown from (1), (2), and (3) that β can be obtained from the equation

$$\frac{hL^2}{2\beta} = (1\text{-}r\text{-}t)L + 0.5h(L^2 - \sum_i n_i^2/m_i) + (r\text{-}h/4)\sum_j l_j j^2 + \sum_i n_i^2\,[t + 9(1\text{-}t)/m_i]/16 + \frac{9r}{16m_i}\sum_{i \neq i'} n_i n_{i'} \frac{p_{ii'}}{m_i m_{i'}}, \quad (4)$$

where L is the total number of lactations in the proof, l_j daughters have had exactly j lactations, n_i daughters are milked in year i, m_i mature cows (not being daughters) are milked in year i, and $p_{ii'}$ mature cows are milked in both years i and i'.

Further assumptions were needed in order to reduce (4) to a simplified expression for β. These included using estimates of some of the parameters, thus leading to an estimate of β. The added assumptions were

(iv) that the number of mature cows (not daughters) in the herd is the same every year, and that this number is 40, and

(v) that estimates of the parameters are $r = .45$, $h = .25$, and $t = .20$.

The expression for β then reduced from (4) to

$$1/\hat{\beta} = 2.8/L + 1/2 + \frac{3.1}{L^2}\sum_j l_j j^2 + \frac{.1775}{L^2}\sum_i n_i^2 + \frac{.0506}{L^2}\sum_{i \neq i'} n_i n_{i'}\, p_{ii'}\,/40 \quad (5)$$

The assumed value of n_i is 40; thus, $p_{ii'}/40$ when $(i-i') = 1$, is the fraction of the mature cows in a herd that are milked in two consecutive years. From the New Zealand Dairy Board (5), an estimate of this fraction was obtained as 70%, giving values for the fraction of .50 and .34 when the difference between i and i' is 2 and 3, respectively. With these values, expression (5) reduced to a function dependent on the number of daughters a sire has, and the pattern of lactations which they have made over the years of the proof. Such an expression could be computed for each individual sire but, from a practical viewpoint, this is quite unfeasible, so further simplification was sought.

Simplifications leading to working values. First, use was made of certain relationships existing among some of the terms already defined, namely

$$\sum_j d_j = N, \text{ and } \sum_j n_i = \sum_j l_j j = L.$$

Two new sets of parameters were defined, dealing with the pattern of culling in a herd: r_2', r_3', and r_4' . . . were defined as the fraction of 2-, 3-, and 4-yr.-olds, respectively, retained in a herd from one year to the next, so that $(1-r_2')$ for example, is the culling rate for two-yr.-olds; p_1, p_2, and p_3 . . . were taken as the proportions of the total number of daughters in the complete proof, which were in the proof at the end of the 1st, 2nd, 3rd . . . yr. of the proof, respectively. These r''s and p's completely specify the culling that has taken place in a herd, and they were substituted into expression (5) in place of the l_j's. Although it is not true that the same culling rates on an age basis do apply in all years of a proof and for all herds, this is an expedient and practical form of simplification.

At this stage, the expressions for $\hat{\beta}$ were considered separately for 1-, 2-, 3-, and 4-yr. proofs. For a 1-yr. proof, the expression is

$$1/\hat{\beta} = \frac{5.9}{L} + .6775, \tag{6}$$

and this can be computed directly for any value of L.

When there are just two years' daughters in a proof

$$1/\hat{\beta} = \frac{12.1}{L} - \frac{6.2}{pL} + .6775 - \frac{.2841 p_1(1-p_1)}{p^2}, \tag{7}$$

where p is the average number of lactations per daughter,

$$\text{i.e., } p = L/N, \tag{8}$$

$$\text{and } p = 1 + r_2' p_1, \tag{9}$$

by the definition of r_2' and p_1. For seven different values of p_1 (fraction of the total number of daughters in the proof that were in it the 1st yr.) ranging from 1.0 to 0.2, and for seven values of r' (fraction of 2-yr.-olds retained in the herd each year) ranging from 0.0 to 0.5, values of p were calculated from (9). For each of these, values of p, $\hat{\beta}$ and N were expressed in terms of L from (7) and (8) and these were then calculated for all values of L from 6 to 45. For any given

values of L and N it was found that the values of $\hat{\beta}$ for different values of p differed only at the third decimal place. The values of $\hat{\beta}$ were, therefore, summarized in a two-way table according to N and L.

This same procedure was followed for calculating $\hat{\beta}$ for proofs spreading over 3 and 4 yr., extending the values of L in these cases to 90 and 150, respectively. The "smoothing" of the tables became progressively a little more difficult, due to the gradations in p (the average number of lactations per daughter) not being so fine as in the 2-yr case. The sets of values for the p's and r's that were used were by no means exclusive, but they covered a wide range, from no culling to total culling, and from the case of assuming all daughters in a proof being in it the 1st yr. to where only a few of them are. No highly irregular values of $\hat{\beta}$ appeared.

There were now four tables of $\hat{\beta}$ values, according to values of N and L, dependent on whether the proof was based on 1, 2, 3, or 4 years of lactations. On comparing the tables, it was found that for any specified values of N and L, the maximum differences among the $\hat{\beta}$'s of the four tables was 0.02, so that amalgamating the tables entailed only negligible errors. For the same reason, it seemed unnecesary to carry out the calculations for proofs spreading over more than 4 yr.

Values of $\hat{\beta}$ had been computed in each of the four tables for values of L taken at intervals of one from 6 to 20, intervals of two from 20 to 40, and thereafter at intervals of five. They changed with increasing slowness for large values of L, so the intervals were widened in the amalgamated table, taking two groups as 31-33 and 34-37, and subsequently grouping round L-values of 40, 45 . . . etc. Beyond 82, the L's were grouped by 15's, centering on 90, 105, ... A similar type of grouping was used for the daughter numbers, starting at 25; by one's up to 25, then 26-28, 29-31, and thereafter by 5's, centering on 35, 40, ... These groupings seemed favourable; beyond 30, lactation numbers assume much importance only in multiples of five, and beyond 25 the importance of daughter numbers can not be assessed by individual ones. The result was a comparatively smooth table without any undue repetition of terms.

Consideration of assumptions. It is interesting to consider the assumptions used in arriving at these results. The values of 1/3 in assumption (i) is taken from the New Zealand Dairy Board's Annual Report (5); although assumption (ii) is in direct negation of (i), the fraction would, at most, be small and contribute little to the final result. Assumptions (iii) and (iv) are not true in practice, but practical necessity demands some simplifications, and these are not unreasonable. The average herd size in New Zealand is 60 cows, and although 40 might, therefore, be a little high for the average number of mature cows, a smaller value has little effect on the factors; using 25, decreases them by about 3%. The values used for r and h are in line with those in general use; subsequent work of Castle and Searle (1) has shown that more appropriate values might be $r = .60$, and $t = .10$, but the effect of these changes would be small. Using a constant value for p_{iw}/m_i for all herds has the same weaknesses as assumptions (i) and (iv), but

TABLE 2

Extract from table of factors used for converting difference from expectancy to an index

Number of daughters in progeny-test *(N)*	Number of records in progeny-test *(L)*																			
	6	7	8	9	10	12	14	16	18	20	25	30	35	40	45	50	60	70	80	90	100
6	.30	.31	.32	.33	.34	.36	.37	.38	.39	.40	.41										
7		.33	.34	.35	.36	.37	.38	.39	.40	.41	.43										
8			.35	.36	.37	.39	.40	.40	.42	.43	.45	.47									
9				.37	.38	.40	.41	.42	.43	.44	.46	.48	.50								
10					.39	.41	.43	.44	.45	.46	.48	.50	.51	.52							
12						.43	.45	.46	.47	.48	.50	.51	.53	.54	.55	.56					
14							.46	.47	.49	.50	.52	.53	.55	.56	.57	.58					
16								.48	.50	.51	.53	.55	.56	.57	.58	.59	.61				
18									.50	.52	.54	.56	.57	.58	.59	.60	.62	.63			
20										.51	.55	.57	.59	.60	.61	.62	.63	.65	.66		
25											.55	.58	.61	.61	.62	.63	.65	.66	.67	.68	
30												.57	.62	.63	.64	.65	.67	.68	.69	.70	.71
35													.59	.63	.65	.66	.67	.69	.70	.71	.72
40														.60	.63	.66	.69	.70	.71	.71	.73
45															.61	.64	.69	.71	.71	.72	.72
50																.62	.68	.72	.73	.73	.74
Artificial breeding $L/(L+15)$	.29	.32	.35	.38	.40	.44	.48	.52	.55	.57	.63	.67	.70	.73	.75	.77	.80	.82	.84	.86	.87

the only feasible procedure for terms of this nature which vary so much from herd to herd is to use average values, and apply them universally to all herds.

Factors for A.B. bulls. The development of the regressions as given above is for bulls used in natural service in just one herd. Those used in artificial breeding have daughters in many herds and this fact had to be considered in developing the regression factors for such bulls. It was assumed that every daughter is in a different herd, and that the proof is based on just 1 yr.'s records. In many cases this is not true, but it is (or very nearly so) at the time of a bull's first proof, when his first crop of daughters have made their first records. This is probably the most important proof of all for a bull being used in artificial breeding, because his fate will be decided upon it—continued use in the stud or retirement to the meat works.

The mathematical model used was very similar to that used in the case of natural-service bulls, namely,

$$d_{jk} = \mu + \frac{1}{2} S_j + \frac{1}{2} D_{jk} + G'_{jk} + P_{jk} + H_k + e_{jk}$$

where d_{jk} is the record of the j^{th} bull in herd k. The terms in this model are the same as previously except that Y_{ij}, the effect due to year i in the herd in which the j^{th} bull was used, is now replaced by H_k, the effect due to herd k. The effect due to the i^{th} year is not needed here because of assuming the proof is based on just 1 yr.'s records. Since a bull used in A.B. has daughters in many herds, a herd effect term, H_k, is needed; it was not included specifically in the model (3), for one-herd sires having natural proofs, because such proofs are based on daughters all in the same herd, and in this situation the herd-effect is essentially included in the terms $\mu + y_{ij}$. With sires used in artificial breeding, H_k is assumed a random variable with zero mean and variance σ_H^2. Taking $\sigma_S^2 = \sigma_D^2 = \sigma_g^2$, as has been done, assumes that genetic variance among sires is the same as that among cows within a herd, an assumption that may or may not be true. The same parameter values were used as in the natural service case, with the additional one that σ_H^2 is 30% of the total variance, as in Castle and Searle (1).

The difference from expectancy in this situation can be expressed as

$$D_j = \sum_k (d_{jk} - .75\,\overline{m}_{jk})/L - 76$$

where $\overline{m}_{jk}$ is the mature cow average in Herd k. With the assumptions that have been made, the regression of $\frac{1}{2} S$ on D reduces to

$$\frac{L}{L + 15.456}$$

which, for practical usage, can be expressed as

$$\frac{N}{N + 15}$$

where N is the number of daughters (assumed equal to the number of lactations). It is interesting to note that by evaluating the intra-sire regression (for A.B. sires) of daughter average on contemporary average, Henderson in the United States (3) and Robertson in England (6) obtained values $N/(N+12)$ and $N/(N+18)$, respectively.

Extract of table of factors. The accompanying table is an extract of the complete table of the $\hat{\beta}$ values, the regression of the "difference from expectancy" on one-half the sire's additive genetic value. The difference from expectancy is converted to an index by multiplying it by the appropriate value in this table, dependent upon the number of daughters and lactations in the proof. The bottom line of the table shows the factors for A.B. bulls, where it is assumed that the number of daughters equals the number of lactations. As is to be expected, the factors increase at an ever-decreasing rate as the number of daughters, or the number of lactations, or both, increases. At the lower daughter numbers, increasing the number of daughters increases the factor more than does increasing the number of lactations from the same daughters. This effect acts in reverse, but not so strongly, when there are more than about 25 daughters. The added lactations then imply more information about an already-substantial number of daughters and, if this implies an added number of years in the proof, it means there is then more information about the herd level. These trends show that the first importance is to sample the bull (i.e., get a reasonable number of daughters, about 20) and then sample the environment by getting more lactations from them.

These factors are now being used in the preparation of progeny-test proofs in New Zealand, the index $\hat{\beta}$ (*D.F.E.*) being termed a rating.

ACKNOWLEDGMENT

It is a pleasure to thank Miss O. M. Castle for posing this problem and for her help and advice in preparing this paper. The assistance of Dr. C. R. Henderson of Cornell University in setting up the analysis is also gratefully acknowledged.

REFERENCES

(1) Castle, O. M., and Searle, S. R. Repeatability of Dairy Cow Butterfat Records in New Zealand. *J. Dairy Sci.*, **40**: 1277. 1957.
(2) Hazel, L. N. The Genetic Basis for Constructing Selection Indexes. *Genetics*, **28**: 476. 1943.
(3) Henderson, C. R. Cornell Research on Methods of Selecting Dairy Sires. *Proc. New Zealand Soc. Animal Production*. 16th Conf., 69, 1956.
(4) Lush, J. L. *Animal Breeding Plans*. Iowa State College Press, Ames. 1947.
(5) New Zealand Dairy Board. Annual Reports—1950, 1951, and 1956.
(6) Robertson, Alan, and Rendel, J. M. The Performance of Heifers Got by Artificial Insemination. *J. Agr. Sci.*, **44**: 184. 1954.
(7) Sire Survey and Merit Register. New Zealand Dairy Board, annually. 1958.

A COMPARISON OF ORCHARDGRASS-LADINO CLOVER AND ORCHARDGRASS AS PASTURE FOR MILKING DAIRY COWS[1]

F.R. MURDOCK AND A. S. HODGSON
Department of Dairy Science
AND
H. M. AUSTENSON
Department of Agronomy
Western Washington Experiment Station, Puyallup

SUMMARY

This study indicated higher average daily production of 4% FCM and greater returns over concentrate and fertilizer costs from a mixed pasture of orchardgrass and Ladino clover than from orchardgrass heavily fertilized with nitrogen. Differences in production of FCM between the two pastures averaged 5.0, 3.1, and 2.8 lb/cow/day for years 1955, 1956, and 1957, respectively. Likewise, differences in average returns over concentrate and nitrogen costs were \$0.31, \$0.23, and \$0.18/cow/day for the same years. The progressive decline in differences in production and returns between pasture treatments by years appeared to be related to a similar decline in the percentage of clover in the mixed stand. Returns per acre were greater for the grass and clover mixture by \$47.33 in 1955 (a short pasture season) and by \$55.85 in 1956. In 1957, returns from orchardgrass pasture exceeded those from the mixed pasture by \$5.61 per acre. However, 41 more cow days were required per acre to produce this added return from orchardgrass.

Agronomic yield studies of grasses and grass-legume associations have been conducted under many conditions. In general, the grass-legume mixtures have yielded more dry matter per acre than pure grass stands. However, numerous workers (7, 9, 10) have shown greater dry matter production by pure grass stands heavily fertilized with nitrogen. Very little work has been published concerning the relative nutritive value of grass versus grass-legume pastures for ruminants.

Heinemann and Van Keuren (6) and Van Keuren and Heinemann (12, 13) have reported higher average daily gains by beef and more pounds of beef per acre from grass-legume mixtures than from pure grass pastures. In 1954, the pounds of beef produced per acre from Ladino-orchardgrass pasture exceeded beef production from tall fescue by 26% and from orchardgrass by 76%. The average daily gains from Ladino-orchardgrass were 25% higher than from tall fescue and 39% higher than from orchardgrass in the same comparisons. In 1955, the mixed grass and legume stands again produced more beef per acre and higher average daily gains than did the grass pastures. However, the differences were not so great during the 2nd yr. This was attributed to a very marked reduction in the percentage of clover in the mixed stand. The grass plots were fertilized each year with 200 lb. of nitrogen per acre. The grass and legume

Received for publication March 17, 1959.

[1] Scientific Paper No. 1828, Washington Agricultural Experiment Stations, Pullman. Project No. 1269.

plots were fertilized with 125 lb. of nitrogen per acre in 1954 and 100 lb. per acre in 1955.

The Oregon Agricultural Experiment Station (1) has similarly reported more beef production per acre from grass and Ladino clover than from all-grass pastures heavily fertilized with nitrogen. The monetary returns per acre were also greater for the grass-legume pasture.

McVey and Mott (8) obtained more beef production per acre from a mixture of bluegrass and birdsfoot trefoil than from bluegrass fertilized with 120 lb. of nitrogen per acre.

Blaser *et al.* (4) found lower daily weight gains of beef grazing orchardgrass or tall fescue fertilized with nitrogen than of animals grazing the same grasses with Ladino clover and not fertilized with nitrogen. The weight gains per acre were slightly higher for the animals grazing the pure grass pastures, but the differences were not great enough to pay for the nitrogen fertilizer used. The yearly rate of fertilization was 216 lb. of nitrogen per acre.

In a three-year study, Seath *et al.* (11) found that orchardgrass-Ladino pasture ranked first in monthly persistency of milk production (98.3%), followed in order by Kentucky 31 fescue-Ladino (89.2%), Kentucky bluegrass–Ladino (87.2%), and a straight stand of Kentucky 31 fescue (81.4%). The straight fescue was fertilized with 200 lb. of ammonium nitrate yearly. Under these conditions, the fescue alone produced the highest yearly yield of TDN per acre (2,327 lb.); whereas, the three other pastures yielded about the same, varying from 1,726 to 1,789 lb. per acre.

Wagner (14) has shown that Ladino clover fixed on the average more than 150 lb. per acre annually of utilizable nitrogen when grown with orchardgrass or tall fescue.

EXPERIMENTAL PROCEDURE

The primary objective of this experiment was to compare milk production of cows from orchardgrass–Ladino clover pasture with that from an all-orchardgrass pasture liberally fertilized with nitrogen.

Pasture establishment and fertilization. In the fall of 1954, three replicated plots each of orchardgrass–Ladino clover and orchardgrass were established for this experiment. Each plot was 0.94 acre. Akaroa orchardgrass was sown at the rate of 12 lb. per acre in the grass plots and 10 lb. per acre in the grass-legume association. Certified Ladino clover was sown at the rate of 2 lb. per acre in the latter. The land on which these seedings were established was Puyallup fine sandy loam. It had been clean-cultivated for 2 yr. to eradicate previous plant species.

Mixed fertilizer (10-10-5) was applied uniformly at the rate of 350 lb. per acre at seeding time. The subsequent fertilizer treatments by years are shown in Table 1. In 1955 and 1956, the P_2O_5 and K_2O were split equally between a spring and fall application. In 1957, only a spring application was made because the project was concluded at the end of the grazing season.

Nitrogen was applied to the orchardgrass plots at the rate of 35 lb. N per application. The first application was in early spring and subsequent appli-

TABLE 1

Pounds of N-P_2O_5-K_2O applied per acre by years

Treatment	1955	1956	1957
Orchardgrass	120–130–65	210–125–62	210–64–32
Orchardgrass–Ladino clover	0–130–65	35–125–62	35–64–32

cations were made after each grazing. No nitrogen was applied to the grass-legume plots in 1955. However, in 1956 and 1957, 35 lb. of N were applied per acre to the grass-legume plots in early spring. The nitrogen was applied as ammonium nitrate by means of a mechanical spreader.

All plots were irrigated by sprinkling as needed to supply adequate moisture.

Animal and pasture management. The first cutting of all plots was harvested for silage in the spring of 1955 because of the preponderance of annual weeds. Therefore, the pasture phase of the experiment was not started until July of that year. All plots were strip-grazed throughout the remainder of that season and, also, during 1956 and 1957.

Adjacent plots of each pasture treatment were grazed simultaneously by two groups of lactating purebred Holstein cows during the 3 yr. However, the grazing management was varied slightly between years.

During 1955, two groups consisting of six cows each were paired as nearly as possible according to age, stage of lactation, and milk and fat production. The two groups were assigned at random to the two pasture treatments. Strips of fresh pasture (subplots of approximately 0.08 acre in size) were made available for grazing each day by means of a movable electric fence. Thus, 12 days were required to graze each pair of plots and 36 days to complete a grazing cycle. The cows were not excluded from the previously grazed subplots and were allowed to graze them until moved to the next plot.

At the end of each grazing cycle the animal groups were reversed in regard to the pasture treatments. Two and two-thirds grazing cycles were completed during 1955.

The 36-day grazing cycle used during 1955 was too long and the forage became too mature between grazing periods. Therefore, the size of the daily subplots was increased in 1956, so that only nine days were required to graze each plot and 27 days to complete a cycle. The length of the first grazing cycle was shortened to 16 days in order that all plots would be grazed at a more desirable stage of maturity. In addition, the electric fence was moved to provide fresh pasture twice daily instead of once daily, except for the first day on each plot. A full day's grazing was allowed on the first setting to avoid severe tramping of a restricted area.

Two groups of paired milking cows were used during 1956 in the same type of change-over design used in 1955. The number of cows in each group was changed at times during 1956, to adjust the grazing load to available forage. However, equal numbers were pastured on each treatment and the balance was maintained between groups as nearly as possible. Five and two-thirds grazing cycles were completed during 1956.

Under the fertilization program previously indicated, the total forage dry matter production of the orchardgrass plots during 1956 was significantly greater than that of the grass and clover plots (Table 2). Likewise, the amount of uneaten forage was considerably greater on the grass plots. Therefore, the experimental design was changed during 1957 to measure differences in total milk production per acre when both types of pasture were utilized to approximately the same degree.

Lactating cows were paired as in previous years, but only to the extent of the number grazing the grass-clover plots. Additional cows were added and removed as needed to approximate optimum pasture utilization. Also, animal groups were not reversed in regard to pasture treatments in 1957, but each group remained on the assigned treatment throughout the grazing season.

During all three pasture seasons, the cows were confined to the pastures night and day, except for approximately 2 hr. night and morning while they were at the barn for milking.

Changes in animal weights were ascertained in each of the 3 yr. During 1955 and 1956, the averages of two consecutive daily weights were determined at the beginning and end of each grazing cycle. The net weight gain or loss was then derived for both treatments from these average weights. In 1957, initial and final weights were determined by averaging three consecutive daily weights as animals were started and removed from the experiment.

Concentrate feeding. A simple concentrate mixture containing 15% crude protein was fed at the rate of 1 lb. per 7 lb. of 4% FCM. Adjustments in rate of concentrate feeding were made every six days during the 1955 season and at nine-day intervals during 1956 and 1957.

Observations and analyses. Milk weights for each milking were recorded to the nearest 0.1 lb. Composite milk samples were tested for butterfat at six-day intervals during 1955 and at nine-day intervals during 1956 and 1957. Weights of concentrates fed and refused were recorded to the nearest 0.1 lb. The amounts of fertilizer applied in pounds and dates of application also were recorded. Representative samples of concentrates fed were analyzed for proximate composition by standard A.O.A.C. procedures (2).

Dry matter yields of the pastures were computed from mowed samples taken from successive strips before the cows were allowed to pasture. After the cows were moved to the next replicate, comparable strips were mowed again to determine the amount of dry matter apparently consumed. The Gravely mower used to make these clippings left very little trampled forage after grazing.

The percentages of grass and clover in both pasture treatments were determined on a dry weight basis from hand separations of samples of clipped forage taken for dry matter yields.

Significances of differences in production of 4% FCM between pasture treatments were determined on the data from four grazing cycles during 1956, by an analysis of variance according to the methods of Federer (5). Similar statistical analyses could not be applied to other cycles in the 1955 and 1956 seasons because of experimental design.

RESULTS AND DISCUSSION

The dry matter yields of the pastures and the dry matter consumption by the grazing animals are summarized in Table 2. Correlations of dry matter yields, dry matter consumption, and milk production are being reported in another publication (3).

Variations in the percentage of clover in the stands and the weighted averages for the 3 yr. are shown in Table 3. A yearly decline in the percentage of clover in the mixed stand is very evident. Differences in dry matter production between the grass and the grass-clover appear to be related to the yearly decline in the clover stand and a concomitant decline in nitrogen fertility of the mixed stand.

A nitrogen deficiency became apparent in the orchardgrass-Ladino plots after the first grazing cycle in 1957. This was evidenced by the yellowish color of the grass and reduced rate of growth as compared to grass in the nitrogen fertilized plots. Data in Table 2 indicate higher dry matter yields of both treatments in 1957 than in 1956, in spite of the nitrogen deficiency apparent in the grass-clover plots. These yearly differences were the result of differences in spring growing conditions. Spring forage production was low in 1956, following a long, severe winter, but very abundant in 1957 when growing conditions were very favorable.

The consumption of dry matter in 1955 was significantly higher on grass and clover than on grass alone, even though there was no significant difference in the total dry matter production between treatments. Dry matter production in 1956 was significantly higher for the pure grass plots, but there was no apparent difference in consumption between the two treatments. In 1957, both the dry matter production and the dry matter consumption were significantly higher for the pure grass treatment. In all 3 yr., the percentages of available dry matter consumed by the grazing animals were higher for the orchardgrass-Ladino treatment than for the pure grass treatment. These differences indicate that the grass and clover combination was more palatable.

As has been previously indicated, the rate of stocking was varied between treatments during 1957 so that the available forage from both treatments would be used to the same extent. That this was nearly accomplished is indicated by 67 and 70% consumption of available dry matter from the orchardgrass and orchardgrass-Ladino pastures, respectively.

Variations in the average production of FCM by two groups of cows grazed alternately on orchardgrass-Ladino clover and orchardgrass pastures during 1955 and 1956 are shown graphically in Figures 1 and 2. An increase in milk production is evident when either group was changed from grass pasture to the mixed grass and clover. Each bar in the graph represents the average production by the group while grazing one plot. Three bars represent a complete grazing cycle.

Inasmuch as the experimental design was changed during 1957 so that unequal numbers of animals were pastured on the two treatments, it was not possible to present the production data graphically for that year in the same manner as indicated above.

TABLE 2

Dry matter yields and apparent dry matter consumption of grazing animals by years

	1955			1956			1957		
	Orchard-grass	Orchard-grass–Ladino clover	Difference	Orchard-grass	Orchard-grass–Ladino clover	Difference	Orchard-grass	Orchard-grass–Ladino clover	Difference
D.M. production/acre *(lb.)*	4,090	4,420	330	8,430	7,160	1,270**	9,860	8,050	1,810**
D.M. consumed *(lb.)*	3,200	3,770	480**	5,430	5,280	150	6,590	5,690	900**
D.M. consumed *(%)*	78	85		65	74		67	70	

** Statistically significant at the 1% level.

TABLE 3

Percentage of clover in stands of orchardgrass and orchardgrass–Ladino clover pasture by years (percentages on oven-dry basis)

	1955		1956		1957	
	Orchardgrass	Orchardgrass–Ladino clover	Orchardgrass	Orchardgrass–Ladino clover	Orchardgrass	Orchardgrass–Ladino clover
Range *(%)*	0–4	43–53	0–3	22–39	0–5	3–26
Weighted av. *(%)*	2	47	0	30	3	11

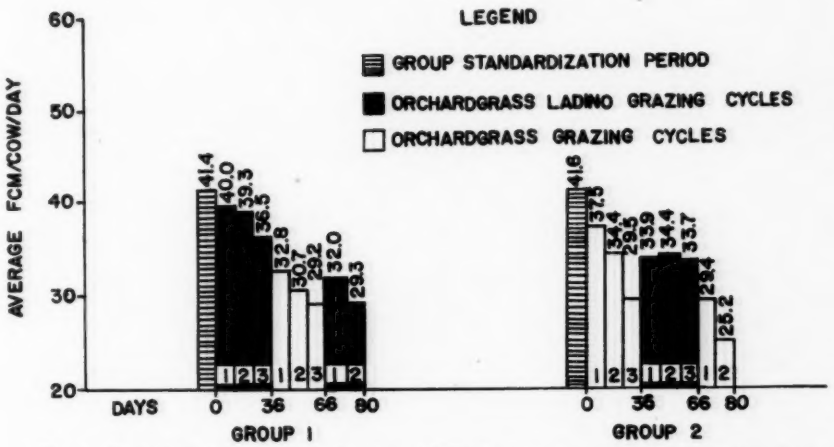


FIG. 1. Variations in average production of FCM by cows on orchardgrass–Ladino clover and orchardgrass pastures during the 1955 season. (Numbers in bars denote successive plots grazed within each pasture cycle.)

An analysis of variance was made on a portion of the milk production data during 1956, to test the significance of differences in milk production between cows while grazing orchardgrass-Ladino clover and orchardgrass alone. Data from the first and last grazing cycles could not be used in this analysis, because the number of days and the number of animals used were not the same as in the intervening cycles. Also, the data from the first plots grazed after each reversal were not used, since these were considered as change-over periods. The period covered by this analysis was from May 30 through September 15 and involves the data from four complete grazing cycles and three group reversals under each treatment. There were five milking cows per group during this period.

The results of this analysis are in Table 4. The greater production of 4% FCM by the cows while grazing orchardgrass–Ladino clover was very highly significant ($P = < 0.0005$).

TABLE 4

Analysis of variance to test the significance of differences in production of 4% FCM by cows grazing alternately orchardgrass–Ladino clover and orchardgrass alone, from May 30 through September 15, 1956

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	39	782,237.46	
Periods	1	169,572.49	
Cows within periods	18	517,343.38	
Subperiods	2	46,765.75	
Treatments within periods	2	36,786.03	
Treatments	1	33,744.49	33,744.49**
Treatments × periods	1	3,041.54	
Error	16	11,769.81	735.61

** $P = < .0005$.

In comparing the milk production and forage production and consumption data by years, it is interesting to note that during 1956 cows produced more milk while grazing the grass-clover mixture, in spite of the fact that more total forage dry matter was consumed from the grass plots. However, differences in dry matter consumption under the two treatments were not significant during that year.

Summaries of the production of 4% FCM and of the economic returns from orchardgrass–Ladino clover and orchardgrass pastures for the 3 yr. are in Table 5. The summaries for 1955 and 1956 do not include that portion of the data applicable to the change-over periods as previously described. Inasmuch as each animal group was confined to one pasture treatment throughout 1957, the data from all plots were included in the summary for that year.

Average daily production of FCM per cow while grazing orchardgrass–Ladino clover exceeded that from cows while grazing orchardgrass during all 3 yr. The differences in FCM production between the two pasture treatments were 5.0, 3.1, and 2.8 lb. per cow per day for the years 1955, 1956, and 1957, respectively. Similarly, the average returns from grass and clover pasture exceeded those from grass alone by \$0.31, \$0.23, and \$0.18 per cow per day for the 3 yr.

The average returns per acre from the orchardgrass-Ladino plots exceeded returns from orchardgrass during 1955 and 1956, but fell slightly below the grass

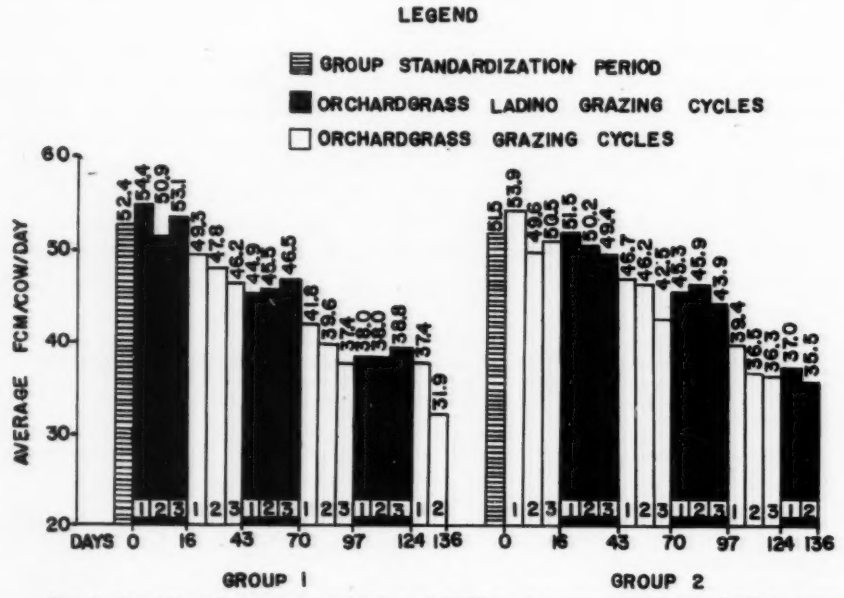


FIG. 2. Variations in average production of FCM by cows on orchardgrass–Ladino clover and orchardgrass pastures during the 1956 season. (Numbers in bars denote successive plots grazed within each pasture cycle.)

TABLE 5

Summary of 4% FCM produced, concentrates fed, ammonium nitrate used, and economic returns per acre from orchardgrass and orchardgrass–Ladino pasture by years

Item	1955			1956			1957		
	Orchard-grass	Orchard-grass–Ladino	Differ-ence	Orchard-grass	Orchard-grass–Ladino	Differ-ence	Orchard-grass	Orchard-grass–Ladino	Differ-ence
Cow days	153	153	.....	248	248	.....	309	268	41
4% FCM *(cwt.)*	46.52	54.18	7.66	105.18	112.78	7.60	118.09	109.93	8.16
Av. FCM/cow/day *(lb.)*	30.4	35.4	5.0	42.4	45.5	3.1	38.2	41.0	2.8
Concentrate consumed *(lb.)*	719	802	83	1,569	1,603	34	1,727	1,618	109
Ammonium nitrate used *(lb.)*	372	.....	372	628	106	522	638	106	532
Milk value @ $4.25/cwt	$197.71	$230.27	$32.56	$447.02	$479.32	$32.30	$501.89	$467.20	$34.69
Concentrate costs @ $70/T	$ 25.17	$ 28.07	$ 2.90	$ 54.90	$ 56.11	$ 1.21	$ 60.44	$ 56.63	$ 3.81
Nitrogen costs @ $95/T	$ 17.67	$ 0.0	$17.67	$ 29.81	$ 5.05	$24.76	$ 30.32	$ 5.05	$25.27
Return over costs of concentrate and nitrogen	$154.87	$202.20	$47.33	$362.31	$418.16	$55.85	$411.13	$405.52	$ 5.61
Av. return/cow/day	$ 1.01	$ 1.32	$ 0.31	$ 1.46	$ 1.69	$ 0.23	$ 1.33	$ 1.51	$ 0.18

plots in 1957. As indicated in Table 2, considerably more dry matter remained on the orchardgrass plots after grazing than on the grass-clover plots. This was particularly true during 1956. Whether this ungrazed dry matter could have been utilized economically is problematical. In 1957, when more cows were allocated to the orchardgrass treatment to use the available forage of both treatments to the same extent, it was found that 41 more cow days per acre were required for the orchardgrass treatment. Additional labor and investment costs, not previously taken into account in calculating the returns shown in Table 5, probably would nullify these apparently greater returns. Thus, it appears that greater production of milk per acre was obtained at the expense of reduced production per cow.

Only the costs of concentrates fed and of the ammonium nitrate applied to the pastures were taken into account in computing the returns from pasture. It is assumed that other costs would be comparable for both treatments, except as noted above during 1957. A value of \$4.25/cwt for 4% FCM was used, because that was the approximate blend price for milk produced in the Puget Sound area during the summer of 1958. The prices used for concentrates and ammonium nitrate are the approximate prices farmers would pay for these commodities in the area.

The average gains and losses in body weight per cow day are shown in Table 6. Inasmuch as weight changes were quite variable between years and between animals within treatments, their significance is questionable. However, during 1956 there was a definite tendency for cows to make greater weight gains while grazing pure orchardgrass than while grazing grass and clover.

TABLE 6

Average gains (+) and losses (−) in body weight per cow day by treatments and years (lb.)

Treatment	1955	1956	1957
Orchardgrass	−0.24	+1.26	+0.28
Orchardgrass–Ladino clover	+0.12	−0.29	+0.10

The formula for the concentrate mixture fed the cows was the same for all 3 yr. The average proximate analysis of the concentrate mixture was 9.6% moisture, 15.0% protein, 3.7% ether extract, 7.0% crude fiber, and 5.5% ash.

One cow bloated on two occasions in 1955 on the orchardgrass-Ladino pasture. The condition was relieved on both occasions by appropriate medication or by means of a stomach tube. These were the only instances of bloat in 1,547 cow days of grazing orchardgrass-Ladino pasture during the 3 yr. of the experiment. Both instances of bloat occurred during the first day's grazing of a new orchardgrass-Ladino plot when the cows had access to a full day's grazing at one setting. It seems likely that the system of strip grazing used (two settings per day) may have aided materially in the control of bloat.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of the following: Dr. T. S. Russell for the statistical analysis of the data presented; V. L. Miller for the chemical analyses of the concentrate samples; and Dr. W. P. Mortensen for advice on fertilizer applications and other technical phases of the experiment.

REFERENCES

(1) Anonymous. Nitrogen—Buy It, or Grow It. *Oregon's Agricultural Progress*, 2: 12. Spring, 1955.

(2) Association of Official Agricultural Chemists. *Official Methods of Analysis.* 8th ed. Washington, D. C. 1955.

(3) Austenson, H. M., Murdock, F. R., Hodgson, A. S., and Russell, T. S. Regression of Milk Production on Forage Production and Forage Consumption. Unpublished data. Western Washington Agr. Expt. Sta., Puyallup. 1959.

(4) Blaser, R. E., Hammes, R. C., Jr., Bryant, H. T., Kincaid, C. M., Skrdla, W. H., Taylor, T. H., and Griffith, W. L. The Value of Forage Species and Mixtures for Fattening Steers. *Agron. J.*, **48**: 508. 1956.

(5) Federer, W. T. *Experimental Design*, pp. 438-443. The Macmillan Co., New York. 1955.

(6) Heinemann, W. W., and Van Keuren, R. W. Irrigated Pasture Studies with Beef Cattle—First Year of Grazing After Establishment of Pastures. Washington Agr. Expt. Sta., *Circ.* 266. 1955.

(7) Holmes, W. The Intensive Production of Herbage for Crop-Drying. III. The Effect of the Continued Application of Nitrogenous Fertilizers to Grassland. *J. Agr. Sci.*, **41**: 64. 1951.

(8) McVey, H. M., and Mott, G. O. Permanent Bluegrass Pastures with and Without Birdsfoot Trefoil. *J. Animal Sci.*, **15**: 1278. 1956.

(9) Mortensen, W. P. Personal communication.

(10) Ramage, C. H., Eby, C., Mather, R. E., and Purvis, E. R. Yield and Chemical Composition of Grasses Fertilized Heavily with Nitrogen. *Agron. J.*, **50**: 59. 1958.

(11) Seath, D. M., Lassiter, C. A., Bastin, G. M., and Elliott, R. F. Effect of Kind of Pasture on the Yield of TDN and on Persistency of Milk Production of Milk Cows. Kentucky Agr. Expt. Sta., *Bull.* 609. 1954.

(12) Van Keuren, R. W., and Heinemann, W. W. Irrigated Pasture Studies with Beef Cattle—Second Year of Grazing. Washington Agr. Expt. Sta., *Circ.* 288. 1956.

(13) Van Keuren, R. W., and Heinemann, W. W. A Comparison of Grass-Legume Mixtures and Grass Under Irrigation as Pasture for Yearling Steers. *Agron. J.*, **50**: 85. 1958.

(14) Wagner, R. E. Legume Nitrogen versus Fertilizer Nitrogen in Protein Production of Forage. *Agron. J.*, **46**: 233. 1954.

A COMPARISON OF THE RELATIVE EFFICIENCY OF THREE PASTURE UTILIZATION SYSTEMS

C. H. GORDON,[1] O. J. HUNT,[2] G. R. MOWRY,[3] AND W. R. HARVEY[4]
Agricultural Research Service, USDA, Beltsville, Maryland

SUMMARY

Utilization of pasture forage by rotational grazing, strip grazing, and soiling, and the effect of these systems on milking dairy cows, was compared at Beltsville in 1954-56. Forage was produced on improved pasture typical of the area. Forage utilized per acre was measured as calculated TDN furnished to milking cows plus TDN removed as excess forage. Effect of the systems on the cows was measured by seasonal milk production and live weight changes.

Average amounts of forage utilized per acre were essentially the same for the two grazing systems. These values were significantly greater for both grazing systems than for the soiling system.

Neither milk production nor live weight changes per cow were significantly affected by the method of providing forage.

The legume content of forage from all areas was rapidly reduced during an extended dry period and remained low during the remainder of the experiment.

Conventional rotational grazing was distinctly the most desirable method of utilizing the forage produced under the conditions of this experiment, although it has been recognized that production of taller, more intensive crops and/or more wasteful management of rotational grazing, such as understocking or allowing forage to become more mature, will provide conditions offering a distinct advantage to strip grazing or soiling.

Three rather distinct pasture forage utilization systems have developed from pasture investigations, in an attempt to reduce the forage wastes that have typified set-stocking and continuous grazing of high-yielding pasture. Although the systems vary in detail and nomenclature between locations, they may be generally described as: 1. Rotational grazing (also known as paddock grazing), whereby the plots of a divided pasture area are grazed in succession for from five to ten days each. 2. Strip grazing (also known as break grazing, daily rationed grazing, and close folding), whereby a subplot sufficient for one day's grazing is assigned each day. 3. Soiling (also called zero grazing, mechanical grazing, and green chop feeding), whereby the forage is mechanically harvested and fed to cows in the barn or dry lot. The relative merits of these three utilization systems are, as yet, matters of no general agreement. Comparisons under a wide variety of situations will be required to assess their values with certainty.

Interest in strip grazing was initially stimulated by reports from Great Britain. Holmes *et al.* (9) in 1950 and Waite *et al.* (23) in 1952 reported marked increases in pasture yields in terms of cow-days, milk, and calculated starch equivalents from strip grazing as compared to rotational grazing. The greater efficiency resulted from the smaller acreage requirement of the strip grazing group. However, additional reports by the same authors, (10) and (24), respectively, showed the rotationally grazed group to have obtained an appreciably higher dry matter intake, indicating that the need for additional acres may have

Received for publication April 27, 1959.

[1] Dairy Cattle Research Branch, [2] Crops Research Division, [3] Farm Electrification Research Laboratory, [4] Biometrical Services.

been less real than apparent. Similar increases in milk yield per acre through the reduced grazing wastes of strip grazing were reported by Procter *et al.* (17, 18).

Soiling has been practiced in this country and abroad for many years. However, soiling has been confined to specialized annual crops during much of this period and regarded only as a supplement to grazing. A report by Graves *et al.* (5) appears to have been one of the first direct comparisons of soiling and grazing the same crop. Their data indicated a somewhat greater yield of TDN for dairy cows from grazing. At about the same time, Tretsven (21) observed no material reduction in acreage requirement, by using a combination of soiling crops when good irrigated pasture was available.

Numerous studies of the relative value of these utilization systems recently have been reported. Ittner, Lofgreen, and Meyer (11) and Meyer, Lofgreen, and Ittner (14) have reported similar gains per steer under strip and rotational grazing of alfalfa, but markedly higher beef yields per acre from strip grazing. Gullickson and Wilcox (6), Henderson, Cobble, and Cook (8), and Hale *et al.* (7) also have reported marked increases in milk or meat per acre from soiling, when compared to rotational or strip grazing, production per animal being essentially the same. However, Richard *et al.* (19) observed a greater gain per steer, as well as more efficient forage utilization from soiling as compared to strip or rotational grazing.

Experiments in which neither milk production per acre nor per cow were significantly different between utilization methods also have been reported during this period [Brundage *et al.* (2), Gullickson and Wilcox (6), Smith and Keyes (20), McMeekan (13), and Porter and Skaggs (16)].

These seemingly contrary results seem to indicate that a factor(s) other than utilization systems has been operative. The differences can not be entirely reconciled on the basis of crop species, since some species, notably alfalfa and smooth bromegrass, are common to both types of results. On the other hand, it seems quite possible that differences in stocking rate may have confounded main effects to some extent, since, in the work cited, except that of Richard *et al.* (19), higher yields per acre have been associated with higher stocking rate rather than with higher yields per animal. Ivins, Dilnot, and Davison (12) have pointed out that the portion of total pasture forage consumed in excess of the requirements to meet the potential productive ability of grazing animals will result in luxury consumption which is not reflected in additional production. McMeekan (13) has also concluded that of the major factors affecting efficiency of pasture conversion on a per-acre basis, stocking rate is by far the most powerful weapon. Thus, differential stocking rates may represent unrecognized treatment differences.

This experiment was initiated to determine the relative merits of rotational grazing, strip grazing, and soiling for milking herds being fed from improved pastures typical of the Beltsville area. These systems were compared on the following basis: (a) effect on the total nutrients utilized per acre; (b) effect on the production of milking cows; (c) effect on the relative needs of the cows for grain supplements; (d) effect on the grass-legume ratio in the sod.

EXPERIMENTAL PROCEDURE

The experimental area consisted of 12 one-acre plots on which an orchardgrass-Ladino clover pasture had been established in 1953. The utilization systems were randomly assigned to the three plots within each of four blocks. Additions of commercial fertilizer were uniform among treatments, but variable from year to year, depending on the apparent requirements of the sod (Table 1). Manure was applied to the soiling plots to compensate for the lack of manure from grazing animals.

The four plots available to each treatment were used rotationally. The grazing groups were moved from plot to plot concurrently, at intervals of from five to ten days; whereas, soiling harvests were scheduled independently, depending on the availability of sufficient forage for mechanical harvesting. Forage in excess of the needs of experimental animals was removed as hay or silage and credited to the proper system, according to its estimated TDN content (15). Thus, the number of times each plot was grazed or harvested depended on its relative position in the rotation and whether it was soiled or grazed. Each plot was managed in a way thought to be most advantageous to its particular system, and all were grazed or harvested four or five times each year.

The forage available on each plot previous to grazing or soiling was estimated by cutting sample strips of forage by the method outlined by Wagner *et al.* (22). The available dry matter was also expressed as estimated TDN, to facilitate comparison with the TDN obtained. The dried strip samples were used for estimating the percentage botanical composition of the crop.

Portable electric fences were moved daily in conjunction with the strip grazing treatment. Such fences were also used when necessary to protect parts of the rotationally grazed plots for mechanical harvesting. Improvement of fencing materials developed during the experiment have previously been reported (4)

The value of these plots for supporting milking dairy cows was measured by the TDN requirements of the cows which utilized them. Four milking cows were assigned to each system. Cows were chosen from a group that had calved 2 to 4 mo.

TABLE 1
Fertilizer applications

Application date	Method and areas	Fertilizer type	Pounds per acre
April, 1953	Band seeding, all plots	0–14–14	500
October, 1953	Broadcast, all plots	0–14–14	500
March, 1954	Broadcast, all plots	0–10–20	300
October, 1954	Broadcast, all plots	0–10–20	300
February, 1955	Broadcast, soiling plots	Manure	10,000
March, 1955	Broadcast, all plots	0–10–20	300
June, 1955	Broadcast, all plots	33–0–0	200
October, 1955	Band seeding, all plots	0–20–0	300
February, 1956	Broadcast, soiling plots	Manure	10,000
March, 1956	Broadcast, all plots	0–0–50	240
May-August, 1956	Broadcast, all plots in three applications	33–0–0	390

previously, so that animals usually remained on their assigned system throughout the season. Cows were assigned to treatments at random, from outcome groups of similar production level, live weight, breeding date, etc.

Supplemental feeding was generally at a rather low rate, with some year-to-year differences. In 1954, one Holstein and one Jersey cow under each management treatment received no grain supplement; whereas, the other Holstein and Jersey in each group received 1 lb. of grain per day for each 2 lb. of FCM produced above 24 lb. per day. During 1955 and 1956, all cows received 2 lb. of grain per day, regardless of milk production. The grain mixture used contained 16% crude protein. Hay was fed only during the first week or two of the pasture seasons.

TDN required by experimental animals was determined by the method recommended by the Joint Pasture Committee (1). The milk produced was weighed at each milking. A composite of aliquot samples of milk from four consecutive milkings was tested every ten days for butterfat content by the Babcock method. All feeds fed and refused (excepting grazed forage) were weighed. Dry matter content of the forage fed and refused for the soiling treatment was determined daily, whereas that for supplemental feeds was usually determined each ten days. The TDN content of the supplemental feeds was based on Morrison's estimates of the digestibility of similar feeds (15).

All grazing cows were on pasture continuously with the exception of two, 2-hr. periods each day, when they were brought to the barn for water, supplements, if any, and milking. Cows assigned to the soiling treatment were housed in a stanchion barn and individually fed green forage to the limits of appetite twice daily. The green forage was harvested daily with a flail type harvester and a trailer especially equipped to reduce field losses of harvested material.

When experimental plots failed to produce sufficient forage to support the cows assigned to them, the cows were continued on the same feeding system by utilizing similar nonexperimental forages. Occasionally, however, no suitable forage was available and the feeding systems were suspended for short periods. The continuity of the feeding systems is shown in Table 2.

TABLE 2

Dates of experimental and nonexperimental management of cows

Year	Management	Dates first grazing or harvest	Dates last grazing or harvest	Periods of nonexperimental management
1954	Strip grazing	April 15	September 30	Aug. 5–19[a]
	Rotational grazing	April 15	September 30	Aug. 5–19[a]
	Soiling	April 23	September 28	Aug. 5–19[a]
1955	Strip grazing	April 18	October 3	Aug. 16–31[b]
	Rotational grazing	April 18	October 3	Aug. 16–31[b]
	Soiling	April 18	October 3	Aug. 16–31[b]
1956	Strip grazing	April 25	September 18	June 21–July 3[c]
	Rotational grazing	April 25	September 16	June 21–July 3[c]
	Soiling	April 30	September 14	None

[a] All cows barn-fed during this period.
[b] All cows fed hay and permanent pasture during this period.
[c] All grazing cows fed from permanent pasture as a single group during this period.

OBSERVATIONS AND RESULTS

The productivity of all plots was seriously reduced by the abnormal rainfall pattern throughout the 3-yr. period. Very dry periods occurred in each of the pasture seasons. The only year in which the total rainfall was equal to the 26-yr. average was 1955. Even in 1955, however, two very dry periods occurred, with 14.5 in. of the yearly total occurring during an 11-day period in August. The mid-summer drought of 1954 was believed to be largely responsible for the marked reduction in Ladino clover population in all plots. This loss of clover necessitated the addition of nitrogen fertilizer in June, 1955 (Table 1). Band seeding in the sod in October, 1955, failed to re-establish the clover. The initial legume content of about 35% in the spring of 1954 was reduced to about 3% under all treatments by the spring of 1955, and remained at a low level for the remainder of the experiment. Conversely, the grass content increased from about 63 to about 95% and remained at a high level. Weed content increased slightly during this period, but was never a very large part of the spring herbage. The changes observed in botanical composition did not appear to be associated with utilization systems but with the generally dry seasons.

Estimates of the dry matter available from experimental plots have been summarized in Table 3. The amounts of dry matter available to the two grazing groups were remarkably similar throughout the experiment. Significantly greater amounts of dry matter were available to the soiling system. This greater amount of available dry matter may be explained as follows: All forage samples were cut at a uniform height (2 in.), which was characteristic of the mower. Utilization of the crop to a height of less than 2 in. (which could occur under some grazing conditions) would increase utilization of the present growth and reduce the amount available (taller than 2 in.) that would occur as regrowth for the subsequent grazing. On the other hand, leaving a stubble taller than 2 in. (which could occur when mechanically harvested) would reduce utilization of the present growth, and some growth could appear as available at more than one sampling date. Thus, these values represent the sum of amounts available as estimated at a uniform height at intervals through the season, but do not necessarily represent the total amounts produced above this height.

A summary of the utilization of forage also is presented in Table 3. The two grazing systems furnished essentially the same number of cow-days of grazing per acre. The seasonal carrying capacity of the soiling areas was consistently lower than either of the grazed areas. This was particularly true during periods when relatively small amounts of forage were available.

The amount of TDN utilized per acre by animals on the soiling system was significantly less than that utilized by either grazing treatment (Table 3). This relationship is in accord with that observed on carrying capacity. TDN harvested as excess forage was not significantly different among the treatments. The greater amounts of available forage in 1955 were reflected in greater amounts of excess forage, whereas amounts grazed remained relatively constant each year.

The amount of TDN obtained from soiling or grazing, and the amount harvested as excess, have been totaled to obtain an over-all expression of the per-acre

TABLE 3

Availability and utilization of forage under three utilization systems (per-acre basis)

Year and system	Forage available		Cow-days on experiment	Forage utilized (TDN)				TDN grazed or soiled cow-day	Total standard cow-days[d]
						Total			
	D.M.[a]	TDN[b]		Soiled or grazed	Excess[b] harvested	Lbs.	Relative rotational		
	(lb.)			(lb.)			(%)	(lb.)	
1954									
Rotational	3,917	2,711	133	2,261	378	2,644	100	17.0	165
Strip	4,020	2,782	133	1,925	660	2,585	98	14.5	162
Soiling	4,540	3,142	95	1,566	452[c]	2,018	76	16.5	126
1955									
Rotational	4,744	3,283	135	1,906	1,636	3,542	100	14.1	221
Strip	4,615	3,194	137	1,918	1,768	3,686	104	14.0	230
Soiling	5,426	3,755	114	1,547	1,602[c]	3,149	89	13.6	197
1956									
Rotational	3,820	2,643	132	2,198	242	2,440	100	16.7	153
Strip	3,850	2,664	134	2,284	287	2,571	105	17.0	161
Soiling	4,461	3,087	101	1,592	534[c]	2,126	87	15.8	133
3-yr. average									
Rotational	4,160	2,879	133	2,123	752	2,875	100	16.0	180
Strip	4,162	2,880	135	2,042	905	2,947	103	15.1	184
Soiling	4,809[e]	3,328	103[f]	1,568[g]	863[e]	2,431[f]	85	15.2	152

[a] Estimated from sample strips cut previously to grazing or harvest (see second paragraph under Observations and Results).
[b] Calculated from dry matter values at 69.2% TDN, Morrison's *Feeds and Feeding*, 21st ed. (clover-mixed grass hay, green).
[c] Includes credit to soiling treatment for amount harvested in excess of daily consumption.
[d] Calculated at 16 lb. TDN per standard cow-day.
[e] Significantly more than either grazing system at 1% level of probability.
[f] Significantly less than either grazing system at 1% level of probability.
[g] Significantly less than either grazing system at 5% level of probability.

productivity of each system. The lower productivity of the soiling treatment thus expressed is highly significant.

The differences between systems in calculated TDN obtained per animal per day were not significantly different, thus indicating that animals were on a fairly equal plane of nutrition. The general interpretation of the data in Table 3 made by the authors has been that cows on all systems were fed equally well while they were on the respective areas, but that soiling cows utilized less of the available forage. Hence, the carrying capacity was least.

Performance and requirements of cows while on experimental plots have been summarized in Table 4. These data substantiate the fact that the differences in yields per acre (cow-days or TDN) obtained from the utilization methods (Table 3) were not a function of differences in productivity of cow groups, since the daily requirements of cows were quite similar.

The effect of the forage utilization systems upon the cows was observed almost continuously throughout the season, in spite of the rather intermittent utilization of the tester plots (especially the soiling plots). This was accomplished by using nonexperimental areas as a forage source. Single periods of equal length representing the major portion of the season were selected for each treatment as measures of long-term effects of the systems on animal production. These data are presented in Table 5.

Some differences in average initial FCM production resulted from the random assignment of relatively few cows to a relative large number of treatments. This was especially true in 1954, when the number of treatments was doubled by using two grain-feeding levels.

The average observed milk production values per cow per season for utilization systems and years are presented in Table 5. The same values adjusted by covariance for differences in initial production are also presented.

Average values of FCM per cow per day were used in the analysis of covariance because of differing lengths of seasons. A summary of this analysis is presented in Table 7. No statistical significance could be attached to differences between methods of pasture utilization or years or to the year by utilization method interaction. Highly significant differences between years existed before adjustment for initial production level. Differences between utilization systems were not affected by the year-to-year differences in initial production levels.

Average live weight changes over the entire season also are presented in Table 5. A statistical analysis of the average live weight change per day for individual cows failed to show any significant differences due to treatment. Thus, the treatment differences in both milk production and live weight changes could not be safely considered as more than random variability.

Average seasonal milk production values for 1954 under two supplementation regimes are presented in Table 6. Although the average production of cows receiving grain was higher under all systems than that of the nonsupplemented cows, statistical analysis of the data shows these differences to be nonsignificant, and no statistically significant interaction of utilization systems and supplementation existed (Table 7). Cow variability and small numbers (two cows repre-

TABLE 4

Average performance and TDN requirements per cow per day (while on experimental plots)

Year and treatment	Days on experimental area	Performance			TDN requirements			TDN in supplements	Net TDN from pasture
		Live weight maintained	Live weight change	FCM produced	Maintenance	Live weight change	Milk		
		(lb.)							
1954									
Rotational	133	1,114	+.32	39.62	8.7	1.1	12.7	5.5	17.0
Strip	133	1,063	−.41	36.06	8.4	−1.1	11.5	4.3	14 5
Soiling	95	1,112	+.09	33.67	8.7	0.3	10.8	3.3	16.5
1955									
Rotational	135	992	+.19	22.32	7.8	0.7	7.1	1.5	14.1
Strip	137	994	+.31	20.24	7.9	1.1	6.5	1.5	14.0
Soiling	114	940	−.03	23.87	7.6	−0.1	7.6	1.5	13.6
1956									
Rotational	132	897	+.60	27.47	7.2	2.1	8.8	1.4	16.7
Strip	134	962	+.80	24.94	7.6	2.8	8.0	1.4	17.0
Soiling	101	975	+.37	25.59	7.8	1.3	8.2	1.5	15.8
Av. 1954-56									
Rotational	133	1,001	+.37	29.89	8.0	1.3	9.5	2.8	16.0
Strip	135	1,006	+.23	27.08	8.0	0.8	8.7	2.4	15.1
Soiling	103	1,009	+.14	27.71	7.9	0.5	8.9	2.1	15.2

TABLE 5

Effect of forage utilization system on animal production (entire season)

Period		Average FCM production				Average live weight	
		Initial daily level	Season total	Adjusted season total	Adjusted per cow per day[d]	Initial	Seasonal change
		(lb.)					
1954 (160-day)[a]							
4/15–9/21	Rotational	61.1	6,292	5,314	33.2	1,117	+54
4/15–9/21	Strip	52.6	5,518	5,067	31.7	1,148	−69
4/23–9/29	Soiling	47.1	4,913	4,800	30.0	1,117	+14
1955 (168-day)							
4/18–10/3	Rotational[b]	39.9	3,576	3,933	23.4	1,013	+31
	Strip	48.4	3,190	2,990	17.8	1,002	+52
	Soiling	42.9	3,730	3,888	23.1	933	− 5
1956 (139-day)[c]							
4/30–9/15	Rotational	39.0	3,738	4,078	29.3	844	+86
	Strip	38.6	3,397	3,761	27.1	915	+117
	Soiling	38.2	3,341	3,727	26.8	945	+52
Average							
	Rotational	46.7	4,535	4,442	28.6	991	+57
	Strip	46.5	4,035	3,939	25.5	1,022	+33
	Soiling	42.7	3,995	4,138	26.6	998	+20

[a] Includes production for August 5–19, when all cows were on barn feed.
[b] Includes production for August 16–31, when all animals were on pasture and hay.
[c] Treatments continuous except June 21–July 3, when all grazing animals were on non-experimental pasture as a single group.
[d] Adjusted for differences in initial level, analysis of covariance (Table 7).

sented in each average) resulted in low precision for detecting even rather large differences. Since any true differences or interactions appeared too small to be detected by the precision of the experiment, this variable was omitted in the two subsequent seasons.

DISCUSSION

A major advantage that might be expected from soiling is the elimination of grazing wastes. However, whether this is accomplished with a resulting

TABLE 6

Effect of forage utilization system and grain supplementation on production, 1954, pasture seasons

Treatment	Daily FCM production		
	Initial level	Observed average	Adjusted average[a]
	(lb.)		
With grain supplement			
Rotational grazing	64.2	45.9	38.5
Strip grazing	55.8	37.3	33.2
Soiling	48.2	33.2	32.1
Average	56.0	38.8	34.6
No grain supplement			
Rotational grazing	58.0	32.8	27.9
Strip grazing	49.4	31.7	30.1
Soiling	46.0	28.2	27.9
Average	51.1	30.9	28.6

[a] Adjusted for differences in initial level, 3-yr. combined analysis of covariance (Table 7).

TABLE 7
Analysis of variance of daily FCM with adjustments for initial level of production by covariance (mean squares only)

Source of variation	d.f.	Mean square	F.	
Years (Y)	2	92.06	2.36	N.S.*
Treatments (T)	2	4.28	0.11	N.S.
Y T	4	31.64	0.81	N.S.
Grain levels (G) (1954)	1	104.96	2.69	N.S.
G T (1954)	2	16.82	0.43	N.S.
Cows (C: YTG) Cows C: YTG)		23	39.06	

* N.S.—Indicates not statistically significant.

net gain in utilization efficiency depends upon the relative magnitude of the grazing and harvesting wastes. Therefore, if grazing wastes are sufficiently reduced and harvesting wastes remain constant, it becomes impossible to improve utilization efficiency by harvesting.

The higher per-acre production resulting from grazing, as compared to soiling, is viewed as a result of a less wasteful method of utilization, since no more forage was available to the grazing groups and performance per animal per day was essentially the same for all treatments. The magnitude of grazing wastes is closely associated with the type of crop under consideration. Relatively tall-growing and/or mature crops are much more subject to the wastes of trampling and selective grazing than are shorter and/or less mature crops. However, the wastes of soiling the taller crops will be relatively small, since stubble represents a smaller portion of the total crop and selectivity is greatly reduced.

The results and the observations made demonstrated that pasture forage, typical of this area, may be efficiently utilized by well-managed grazing. Wastes from grazing this type of pasture, however, may be considerable, particularly in the flush period of growth if understocking is allowed, or if grazing is delayed until a hay stage of maturity has been reached. It is considered important that in this experiment these difficulties were overcome by varying the area grazed per cow with portable fences and by mechanical harvesting of the forage, which was in excess of grazing requirements and approaching a mature stage.

The similar utilization efficiency of grazing systems is explained on the basis of similar amounts of wasted forage, since amounts initially available were essentially the same. The mere existence of a rotational grazing or strip grazing system has little bearing on the extent of grazing wastes, since overstocking or understocking is not necessarily eliminated by either. It is true that wastes from understocking are less likely to occur in strip grazing, since estimates of the area required can be made more easily on a daily basis than on an eight- to ten-day basis, but the possibility of equal accuracy in estimates is not necessarily precluded.

It is also recognized that strip grazing is likely to reduce the large wastes that result from rotational grazing of crops taller and/or more mature than those used in this experiment. The data indicate, however, that under certain conditions equal efficiency was attained under both systems, with no significant differences in animal response.

REFERENCES

(1) American Society of Agronomy, American Dairy Science Association, American Society of Animal Production, and American Society of Range Management. Pasture and Range Research Techniques. *Agron. J.*, **44**: 39. 1952.

(2) Brundage, A. L., and Sweetman, W. J. Comparative Utilization of Alfalfa–Bromegrass Pasture Under Rotational and Daily Strip Grazing. *J. Dairy Sci.*, **41**: 1777. 1958.

(3) Brundage, A. L., Sweetman, W. J., Hodgson, H. J., and Bula, R. J. The Utilization of Smooth Bromegrass (Bromus inermis) Under Rotational and Strip Grazing Systems of Pasture Management. *J. Dairy Sci.*, **39**: 280. 1956.

(4) Campbell, L. E., Mowry, G. R., and Hartsock, J. G. Efficiency Factor in Electric Fence Operation. *Agr. Eng.*, **37**: 184. 1956.

(5) Graves, R. R., Dawson, J. R., Kopland, D. V., and Moseley, T. W. Feeding Value for Milk Production of Pasture Grasses when Grazed, when Fed Green and when Fed as Hay or Silage. USDA, *Tech. Bull.* 381. 1933.

(6) Gullickson, T. W., and Wilcox, C. L. Soilage or Rotational Grazing. *Minnesota Farm and Home Sci.*, **13**: No. 2, 18. 1956.

(7) Hale, W. H., Scholl, J. M., McWilliams, R. M., Barnes, K., Zimmerman, R., and Burroughs, W. Feeding Green Forage Clippings in Drylot vs. Grazing Brome-Alfalfa Pasture. Iowa State College, Ames, Iowa. FST-131. 1955.

(8) Henderson, B. W., Cobble, J. W., and Cook, H. J. Soilage Feeding of Dairy Cattle. Rhode Island Agr. Expt. Sta., *Bull.* 336. 1957.

(9) Holmes, W., Waite, R., Fergusson, D. L., and Campbell, J. I. Studies in Grazing Management. I. A Comparison of the Production Obtained from Close-Folding and Rotational Grazing of Dairy Cows. *J. Agr. Sci.*, **40**: 381. 1950.

(10) Holmes, W., Waite, R., Fergusson, D. L., and MacLusky, D. S. Studies in Grazing Management. IV. A Comparison of Close-Folding and Rotational Grazing of Dairy Cows on Intensively Fertilized Pasture. *J. Agr. Sci.*, **42**: 304. 1952.

(11) Ittner, N. R., Lofgreen, G. P., and Meyer, J. H. A Study of Pasturing and Soiling Alfalfa with Beef Steers. *J. Animal Sci.*, **13**: 37. 1954.

(12) Ivins, J. D., Dilnot, J., and Davison, J. The Interpretation of Data of Grassland Evaluation in Relation to the Varying Potential Outputs of Grassland and Livestock. *J. Brit. Grassland Soc.*, **13**: 23. 1958.

(13) McMeekan, C. P. Grazing Management and Animal Production. *Proc. Seventh Intern. Grassland Congr.* Wright and Carmon, Ltd., Willington, N. Z. 1956.

(14) Meyer, J. H., Lofgreen, G. P., and Ittner, N. R. Further Studies on the Utilization of Alfalfa by Beef Steers. *J. Animal Sci.*, **15**: 64. 1956.

(15) Morrison, F. B. *Feeds and Feeding.* 22nd ed. Morrison Publ. Co., Ithaca, N. Y. 1956.

(16) Porter, R. M., and Skaggs, S. R. Forage and Milk Yields from Alfalfa Under Three Different Harvesting Systems. New Mexico Agr. Expt. Sta., *Bull.* 421. 1958.

(17) Procter, J., and Hood, A. E. M. The Close Folding of Dairy Cows. Part II. *J. Brit. Grassland Soc.*, **8**: 239. 1953.

(18) Procter, J., Hood, A. E. M., Fergusson, W. S., and Lewis, A. H. The Close Folding of Dairy Cows. *J. Brit. Grassland Soc.*, **5**: 243. 1950.

(19) Richard, R. M., Roubicek, C. B., Rosenblatt, L. M., McAlister, D. F., Stone, R. R., and Pritchard, F. The Comparative Value for Beef Production of Irrigated Alfalfa Harvested by Different Methods. Arizona Agr. Expt. Sta., *Rept.* No. 139. 1956.

(20) Smith, E. P., and Keyes, E. A. Comparison of a One-Day Rotational to a Ten-Day Rotational Pasture Management Plan for Dairy Cows. *Proc. 35th Ann. Meet., West. Div. Am. Dairy Sci. Assoc.* 1954.

(21) Tretsven, J. O. Feeds for Dairy Cattle. Montana State College, Bozeman, Mont., *Bull.* 282. 1933.

(22) WAGNER, R. E., HEIN, M. A., SHEPHERD, J. B., AND ELY, R. E. A Comparison of Cage and Mower Strip Methods with Grazing Results in Determining Production of Dairy Pastures. *Agron. J.*, **42**: 487. 1950.

(23) WAITE, R., HOLMES, W., AND BOYD, J. Studies in Grazing Management. V. Further Determination of the Nutrient Intake of Dairy Cows Under Close-Folding and Rotational Methods of Grazing. *J. Agr. Sci.*, **42**: 314. 1952.

(24) WAITE, R., HOLMES, W., CAMPBELL, J. I., AND FERGUSSON, D. L. Studies in Grazing Management. II. The Amount and Chemical Composition of Herbage Eaten by Dairy Cattle Under Close-Folding and Rotational Methods of Grazing. *J. Agr. Sci.*, **40**: 392. 1950.

SOME FACTORS AFFECTING WEIGHT GAINS OF DAIRY HEIFERS FED ALL-ROUGHAGE RATIONS[1]

M. E. McCULLOUGH AND W. E. NEVILLE, JR.
Georgia Agricultural Experiment Station, Experiment

SUMMARY

Factors affecting weight gains by dairy heifers were studied with 28 heifers on pasture and ten individually fed heifers on either an all hay or grass silage ration. The heifers averaged about 450 lb. in weight. The only factor significantly correlated with gains on pasture was forage dry matter digestibility (r=.797). On pasture, the multiple correlation between average daily gains and dry matter digestibility, dry matter intake, and body weight was .831. Over a 56-day feeding period, the correlations between dry matter intake and average daily gains were .711 for silage and .399 for hay. Correlations between body weight and dry matter intake were .932 for silage and .857 for hay. Multiple correlations between average daily gains, body weight, and dry matter intake were .858 for silage and .463 for hay. Respective average daily gains for pasture, silage, and hay were 1.79, .92, and 1.34 lb. The error of estimate of weight gains in all groups prevents accurate estimate of response from the factors measured and emphasizes the large number of animals required to show significant differences between rations. On an individually fed hay ration, 82, 14, and four animals per treatment were required to show significant differences in average daily gains of .10, .25, and .50 lb. between group means. On silage, 174, 28, and seven animals were required to show the above differences. When the effects of body weight and dry matter intake of silage were removed, the number of animals needed was reduced to 72, 12, and three. The data would appear to indicate that intake was a major factor only on the grass silage.

Live weight gain is the most frequently used measure of the nutritional value of forages for dairy heifers. The adequacy of a feed for growing heifers is measured by the observed body weight gain in relation to a desired rate of gain represented by feeding standards or personal preferences. The general problems of feeding trials with dairy heifers were reviewed by Bailey *et al.* (1) and need not be repeated here. Since it is impossible to feed all forages and all combinations of forage in feeding trials, future work should emphasize principles of forage utilization and experimental efficiency.

EXPERIMENTAL PROCEDURE

The animals used in all trials were Holstein and Guernsey heifers from the station herd. The hay was a good-quality, second-cutting alfalfa, the silage was made from winter oats cut at the pre-bloom stage and ensiled without preservative, and the pasture was a winter temporary pasture consisting largely of oats and rye-grass. The hay was chopped fine and fed twice daily in quantities large enough to insure weighbacks. The silage was fed fresh and was also fed twice daily with weighbacks. All animals were individually fed and ten animals were used per treatment. The 27 pasture observations were taken from an experiment designed to study season-long changes in pasture quality. The data used were

Received for publication January 9, 1959.

[1] Georgia Experiment Station Journal Series No. 353.

from animals which had been on the pasture for a minimum of 6 wk. Intake and digestibility data were determined by the chromogen–chromic oxide technique. Chromic oxide was administered twice daily in capsules, grab samples were collected twice daily and composited for each animal over a five-day collection period. The animals were fed each ration for seven days before the start of the experimental period to allow for adjustment in fill. All animals were weighed in the early afternoon; the animals on hay and silage were weighed at seven-day intervals and the pasture group at 14-day intervals.

Chromogen was determined by the technique of Reid *et al.* (5), chromic oxide by the technique of Schurch (6), and the statistical techniques were those of Snedecor (7).

RESULTS AND DISCUSSION

Factors affecting animal gains. The mean intake and performance data for the three roughages are shown in Table 1 and the relationship between the variables in Figure 1.

TABLE 1

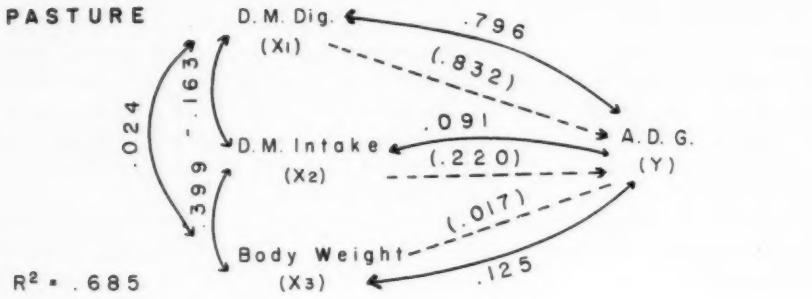
Average body weight, dry matter intake, and gain of heifers consuming the various forages

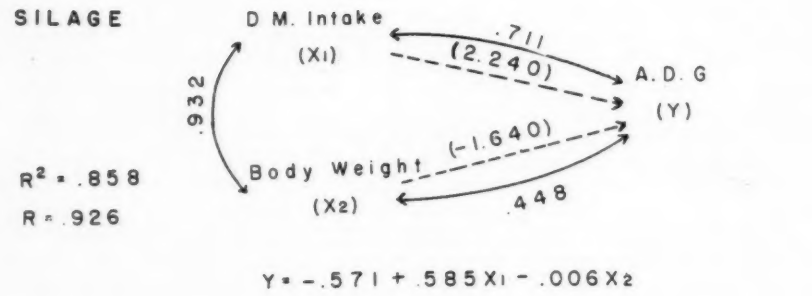
Type of forage	Range in body weight	Mean body weight	Mean dry matter intake	Correlation with body size	Mean daily gain
	(lb.)			*(r)*	*(lb.)*
Pasture	322–662	486	20.22	.399[b]	1.79
Silage	266–622	442	7.40	.932[a]	0.96
Hay	305–685	469	12.08	.897[a]	1.34

[a] Significant at 1% level of probability.
[b] Significant at 5% level of probability.

Body weight. Body weight was highly correlated with dry matter intake for the animals on the rations of hay ($r = .897$) and silage ($r = .932$). An increase in body weight of 100 lb. resulted in an increased dry matter intake of 1.39 lb. of silage or 1.36 lb. of hay. The failure of increasing body weight to show a significant increase in intake for animals on pasture may reflect either the effects of selective grazing or a general tendency for the animals to consume more than adequate quantities of forage on high-quality pasture. This relationship is in accord with the findings of Garrigus and Rusk (3) and Crampton and Purdy (2), but not with those of Martin *et al.* (4), who found an average increase in dry matter intake of 3.5 lb. for each 100-lb. increase in body weight. In no case was body weight significantly correlated with average daily gain, although with silage there was an indication that the larger animals enjoyed an advantage, since body weight accounted for 27% of the differences in average daily gain.

Dry matter intake. Dry matter intake was highly correlated with average daily gains only in the case of the silage, where it accounted for 58% of the variation in gains. It was apparent that the forages presented three different intake conditions. Tests for significance indicated a significant difference between forages for dry matter intake, even after adjustment for differences in average weight of animals. Silage constituted a ration on which it was difficult





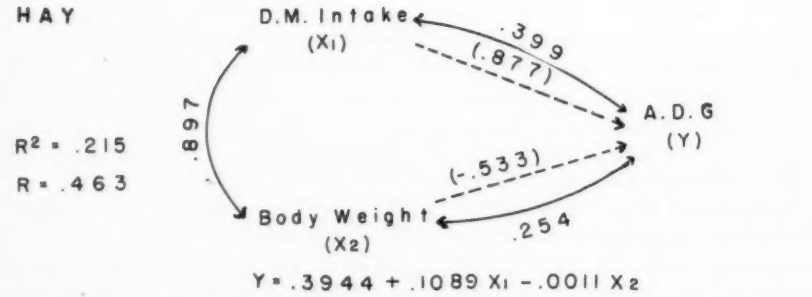


FIG. 1. Factors affecting average daily gain on three types of roughage. (Path coefficients in parentheses.)

for the animals to meet their nutritive requirements, thus giving an advantage to both the larger animals and those with a greater appetite or capacity for the feed. The high-quality pasture apparently represented a forage condition conducive to large intake by even the smaller animals and adequate intakes by those with the smaller appetite or capacity. The hay ration was apparently intermediate between the two.

Differences in average daily gain due to changing forage quality were measured only in the case of the pasture. Dry matter digestibility was highly correlated with average daily gain ($r = .796$) and accounted for 60% of the variation in gains. The data indicated that forage quality is the major factor in determining the adequacy of all-forage rations for dairy heifers. On high-quality forage even small animals are able to meet their nutrient requirements, but on low-quality forages only the larger and older animals have the capacity or appetite necessary to meet their needs. It should be pointed out that further work may result in the finding of factors other than quality as measured by stage of maturity and digestibility which affect the intake of silage. If so, then silage quality must include the effects of ensiling as well as conventional quality measurements.

Duration of trial and animals required for forage evaluation. The data permitted calculations on the effect of length of feeding trials on the reduction in the standard error of average daily gain and an estimation of the number of animals required for evaluating forages of varying magnitudes in quality. These data are shown in Tables 2 and 3. Since all animals were fed the experimental ration for seven days before the start of each experiment, the seven-day weight given in Table 2 was actually made 14 days after the animal was started on the

TABLE 2

Effect of length of trial on standard error of average daily gain

Type of forage	Days on experiment							
	7	14	21	28	35	42	49	56
Pasture	....	.23	....	.24	....	.17	....	.12
Silage	.40	.30	.25	.19	.16	.14	.14	.13
Silage	(Omitting first 21 days)			.41	.22	.14	.15	.13
Hay	.50	.41	.23	.24	.15	.14	.11	.14
Hay	(Omitting first 21 days)			.39	.15	.12	.10	.10

TABLE 3

Estimated number of animals required to evaluate rations

Difference in treatment means	Number of animals required		
	Hay	Silage	
		Effects not removed[a]	Effects removed
(lb.)			
0.10	82	174	72
0.25	14	28	12
0.50	4	7	3

[a] "Effects" refers to body weight and dry matter intake. There was no advantage in this correction on the hay group.

feed. Data in Table 2 indicate that a satisfactory standard error required a 56-day experimental period, although the reduction in standard error was slight after 42 days. A comparison of the data for silage and hay, calculated by using the 21-day weight as the starting point (actually 28 days after the animal was started on feed), indicates that the standard errors are composed of at least two factors. The standard error is reduced by the animal becoming accustomed to the ration and also by a sufficient length of time to overcome the magnification of errors due to small differences in body weight between weigh periods. The latter factor probably operates largely through weighing errors due to small changes in fill which are important only when small differences in body weights are being considered. A further contributing factor may be differences between animals in the acceptance of the rations. This may explain, in part, the seemingly greater accuracy of early weighings of the animals on pasture, since their average daily gain was greater and the weigh periods were at 14- rather than seven-day intervals.

Estimates of the number of animals required to determine significant differences in treatment means of 0.10, 0.25, and 0.50 lb. average daily gain are shown in Table 3. The large numbers of animals required for 0.10 lb. reflect the large standard error in average daily gains and the many factors (largely unknown) which govern the growth of animals. The large reduction in animal numbers resulting from removing the effects of body weight and individual intakes on weight gain in the case of silage indicates the importance of knowing and removing factors affecting weight gain, other than those due to ration, whenever possible. The data also indicate that small differences in forage quality can not be measured with the animal numbers usually available for experimental purposes. Similar data for pasture experiments were not calculated, since pastures vary in quantity as well as quality of forage, although animal numbers similar to those given for hay should be expected when great care is exercised to keep only forage quality as a variable.

REFERENCES

(1) Bailey, G. L., Broster, W. H., and Burt, A. W. A. Experiments on the Nutrition of the Dairy Heifer. II. *J. Agr. Sci.*, **50**: 1. 1958.

(2) Crampton, E. W., and Purdy, T. L. Pasture Studies. XXII. Dry Matter Defecations as an Index of Forage Intake by Grazing Steers. *Sci. Agr.*, **22**: 242. 1941.

(3) Garrigus, W. P., and Rusk, H. P. Some Effects of the Species and Stage of Maturity of Plants on the Forage Consumption of Grazing Steers of Various Weights. Illinois Agr. Expt. Sta., *Bull.* 454. 1939.

(4) Martin, C. M., Brannon, W. F., and Reid, J. T. Relationship of Size of Growing Cattle to Pasture Intake and Its Use as an Index of Palatability. *J. Dairy Sci.*, **38**: 181. 1955.

(5) Reid, J. T., Woolfolk, P. G., Hardison, W. A., Martin, C. M., Brundage, A. L., and Kaufmann, R. W. A Procedure for Measuring the Digestibility of Pasture Forage Under Grazing Conditions. *J. Nutrition*, **46**: 255. 1952.

(6) Schurch, A. F., Lloyd, L. E., and Crampton, E. W. The Use of Chromic Oxide as an Index for Determining the Digestibility of a Diet. *J. Nutrition*, **41**: 629. 1950.

(7) Snedecor, G. W. *Statistical Methods.* 3rd ed. Iowa State College Press, Ames. 1940.

NUTRIENT LOSSES, QUALITY, AND FEEDING VALUES OF WILTED AND DIRECT-CUT ORCHARDGRASS STORED IN BUNKER AND TOWER SILOS

C. H. GORDON, E. A. KANE, J. C. DERBYSHIRE, W. C. JACOBSON, AND C. G. MELIN
Dairy Cattle Research Branch, Animal Husbandry Research Division,

AND

J. R. McCALMONT
Agricultural Engineering Research Division,
USDA, Beltsville, Maryland

SUMMARY

First-cutting orchardgrass was preserved as: 1. Wilted silage stored in a tower silo. 2. Direct-cut, sodium metabisulfite–treated silage, stored in a tower silo. 3. Direct-cut sodium metabisulfite–treated silage, stored in a bunker silo. These silages were prepared in each of two successive years and all silages were sealed with weighted plastic covers. The silages were compared on the basis of storage losses, storage temperatures, chemical quality, feeding trials, and digestion trials. An expression of silage feeding value which combines dry matter intake rate and TDN content has been proposed.

Tower storage of wilted forage and bunker storage of the direct-cut forage were about equally efficient in preserving feed nutrients. Tower storage of direct-cut forage was consistently less efficient, primarily because of higher seepage losses. All silages were of good chemical quality and of about equally high feeding value. The wilted silage, however, contained more butyric acid in both years and was consumed by dairy cattle in significantly greater amounts one year.

The bunker silo, as used in this experiment, was a more suitable storage structure for direct-cut orchardgrass than a tower silo. However, when forages were stored in tower silos, the wilting method was generally more satisfactory than direct-cutting combined with sodium metabisulfite treatment.

Difficulties in obtaining well-preserved and palatable silages from direct-cut, high-moisture hay crops have been repeatedly although not consistently observed. Mitigation of these problems by wilting the crop, as demonstrated by Woodward and Shepherd (11), has been reported by the Vermont and Ohio workers (8, 9), as well as by numerous other investigators. Wilting is now one of the generally accepted ensiling procedures. Improved preservation and/or increased palatability by the use of sodium metabisulfite applied to the unwilted silage has also proven beneficial under some conditions (1, 2, 5). Although both of these procedures have produced some beneficial effects, few direct comparisons between the two methods have been reported. A direct comparison of this type, reported by Cowan *et al.* (2), indicated a marked advantage of bisulfite treatment over wilting for the dry matter preservation of pre-bloom and full-bloom alfalfa, but no advantage for 1/10-bloom alfalfa. However, these results may not provide a practical comparison of methods, since the silages were made in 4- by 8-ft. silos, and dry matter losses of up to 35% were observed in the wilted silage. Other investigations (10, 11), using farm-sized silos, have reported losses of about one-third this amount from wilted silage.

Received for publication June 10, 1959.

The relative value of bunker silos and tower silos can not be accurately assessed from previously published reports. Cowan *et al.* (3) and Gordon *et al.* (4) have reported quantitative evaluations of trench and bunker silos, respectively, but no direct comparisons with tower silos were included in these experiments. A critical comparison of these two silo types demands concurrent use with the same crop. This experiment was, therefore, designed to allow the following direct comparisons: 1. Wilting vs. sodium metabisulfite treatment of an unwilted crop, both stored in tower silos. 2. Tower vs. bunker storage, when both silages were unwilted, treated with sodium metabisulfite, and sealed by the best practical methods available.

EXPERIMENTAL PROCEDURES

The crops used in this investigation were harvested in 1956 (Experiment 1) and 1957 (Experiment 2), and fed during the winters following each harvest.

Experiment 1. Forages from a first-cutting crop of orchardgrass were harvested and stored concurrently by three different methods. All forages were chopped with a forage harvester set for a ⅜-in. cut. The procedures in harvesting and storing the forage were as follows: 1. Field-wilted to an average moisture content of about 70%, stored with no additive in a 10- by 25-ft. concrete tower silo. 2. Not wilted, stored in a 10- by 25-ft. concrete tower silo with 8 lb. of sodium metabisulfite (SMB) per ton of forage added at the blower. The tower silos were alternately leveled and tramped during the filling period. 3. Not wilted and stored in a 15- by 66- by 8-ft. drive-through bunker silo. Sodium metabisulfite (SMB) at the rate of 8 lb. per ton of forage was manually applied to each load of forage after it had been spread in the bunker. Bunker-stored forage was packed with a wheel-type tractor during the filling period.

Filling was started on May 2, when most of the grass heads were visible but not fully emerged from the boot. Rain interrupted the filling operation after two loads of direct-cut forage had been stored in the tower silo. A temporary plastic seal prevented spoilage in this silo until filling was resumed. Filling of all structures was completed during the May 7-11 period.

Bunker-stored silage was sealed May 11 with a neoprene-nylon blanket weighted with a 2-3 in. layer of sawdust. Tower-stored silages were sealed with sheets of polyethylene weighted with nonexperimental forage. Guttering and flow meters were utilized to measure the seepage from each silo.

Each load of stored forage was weighed and sampled for chemical analyses. Silage temperatures during storage in tower silos were measured by thermocouples located at 0.5, 6.5, 12.5, 18.5, and 24.5 ft. above the silo floor. Silage removed for feeding was weighed and sampled daily. Five-day composite samples were analyzed for dry matter (toluene distillation) pH, sugar, carotene, crude protein, ammoniacal nitrogen, and organic acids. Dried 20-day composites were analyzed for the remaining constituents.

The silos were opened and feeding commenced after a storage period of about 6 mo. Feeding values of the silages were estimated with 12 milking cows in a 120-day 3×3 Latin-square feeding trial. Each period consisted of a ten-day change-over and 30-day experimental period.

The rations consisted of the experimental silage, fed to the extent of appetite, and grain fed according to the equalized feeding system described by Lucas (6). The grain ration, composed of cornmeal 44%, ground oats 22%, wheat bran 22%, soybean oil meal 11%, and salt 1%, was fed at a grain : FCM ratio of about 1 : 4.

Data were obtained on a daily individual cow basis for the weights of feeds fed and refused and milk produced. Butterfat content of the milk was determined for each cow from two-day composite samples taken once during each ten-day period. The dry matter content of refused feed was determined at five-day intervals and that of the grain each 20 days. Sampling and analysis of the silages fed have been described.

Dry matter digestibility and TDN content of the silages were determined by the total collection method after the completion of the feeding trial. The same animals and ration assignments (excepting the omission of grain) that were used during the last feeding trial period were extended for the digestion trials. Thus, a randomized block of four replications of three cows each was the design used for digestibility trials.

Experiment 2. The procedures and methods used were generally the same as those used in Experiment 1, with the following exceptions: Filling was accomplished without interruption during the period May 6–10. The tower silos had been enlarged to a height of 35 ft. Thermocouples were located at 3-ft. intervals up to 27 ft. above the silo floor. Thermocouples were also located in the middle of the bunker silo at 1.5, 3.0, 4.0, and 5.5 ft. above the floor, and at 8 ft. from the end of the bunker at 3.0 and 4.0 ft. from the floor. Four thermocouples were distributed transversely at each of the above bunker locations, making a total of 24 points in the bunker. The digestion trials were conducted concurrent with the feeding trial. Three additional cows on all silage rations were utilized for this purpose in a 3×3 Latin-square design.

RESULTS

The chemical characteristics of stored forages (Table 1) within each experiment were very similar, with the exception of a higher dry matter content in the

TABLE 1
Average chemical composition of forages as stored

Constituents[a]	1956 Crop treatments			1957 Crop treatments		
	Wilted in tower	Direct-cut in tower	Direct-cut in bunker	Wilted in tower	Direct-cut in tower	Direct-cut in bunker
Dry matter	30.0	22.2	21.8	30.8	23.8	25.2
Crude protein	12.8	13.6	13.5	13.0	13.4	13.2
Ether extract	3.1	3.4	3.2	2.9	3.5	3.3
Crude fiber	25.9	24.9	24.9	27.8	26.5	26.6
N.F.E.	49.4	47.4	47.9	48.3	46.8	47.5
Ash	8.8	10.6	10.6	8.0	10.0	9.4
Red. sugars	8.6	8.6	8.4	10.5	10.3	10.1
Carotene	163.7	199.3	194.2	205.9	226.4	214.6

[a] All constituents except dry matter and carotene expressed as per cent of dry matter. Carotene expressed as μg/g of dry matter.

wilted forage and a higher ash content in the SMB-treated forages. Wilting apparently reduced the carotene content, particularly in Experiment 1. The most noticeable difference between years in the composition of forages was the higher dry matter content of the 1957 unwilted crops.

Average chemical composition of the silages removed for feeding is presented in Table 2. The higher dry matter content resulting from wilting per-

TABLE 2
Average chemical composition of silages as fed

Constituents[a]	1956 Crop treatments			1957 Crop treatments		
	Wilted in tower	Direct-cut in tower	Direct-cut in bunker	Wilted in tower	Direct-cut in tower	Direct-cut in bunker
Dry matter	29.0	25.4	22.4	31.4	27.4	25.0
Crude protein	14.4	13.5	14.2	13.0	12.5	13.0
Ether extract	3.3	3.5	3.6	3.9	3.5	3.7
Crude fiber	29.5	29.8	28.8	30.4	29.3	28.6
N.F.E.	42.7	43.1	42.5	44.7	45.8	45.5
Ash	10.2	10.1	10.9	7.9	9.0	9.2
Sugar	0.8	2.6	3.0	3.4	4.4	4.7
Carotene	215.6	275.5	236.1	179.4	241.4	215.1
pH	4.2	4.2	4.4	4.2	4.2	4.2
Ammoniacal nitrogen	12.5	10.1	9.7	11.4	8.9	7.5
Butyric Acid	2.1	0.4	0.5	1.2	0.3	0.4
Acetic Acid	1.9	1.2	1.5	1.4	1.3	1.4
Propionic Acid	0.2	0.1	0.1	0.2	0.1	0.1
Lactic Acid	5.2	4.2	3.8	5.8	5.2	5.0

[a] Ammoniacal nitrogen expressed as per cent of total nitrogen. Carotene as $\mu g/g$ of dry matter; all other constituents except dry matter and pH expressed as per cent of dry matter.

sisted through the storage period, although the difference was reduced by an increase in per cent dry matter in the tower-stored unwilted silages. This increase is an expected result of the loss of moisture through seepage.

Higher total reducing sugar and carotene contents characterized the SMB silages in both years. This rather commonly observed effect is of questionable nutritional significance, although the higher sugar content may be indicative of a less intense fermentation. Protein degradation, as indicated by ammoniacal nitrogen values, was quite well controlled in all silages, although it tended to be higher in the wilted silages.

Organic acid concentrations and pH values indicated that all of the tower-stored silages were of excellent quality. However, somewhat greater amounts of organic acid were observed in the wilted than in the SMB-treated silage in both experiments. The wilted silage had a pleasant odor in spite of its higher butyric acid content. Chemical quality of the bunker-stored silage was generally equal to that obtained by tower storage, in both years.

Forage dry matter was most efficiently preserved by wilting and storage in a tower silo, and least efficiently preserved by direct cutting combined with SMB treatment and tower storage (Table 3). Bunker-storage efficiency exceeded that of tower storage for direct-cut forage and was nearly equal to that obtained by wilting and tower storage. A generally higher rate of dry matter recovery for all

TABLE 3
Percentage of stored nutrients preserved for feeding

Nutrients	1956 Crop treatments			1957 Crop treatments		
	Wilted in tower	Direct-cut in tower	Direct-cut in bunker	Wilted in tower	Direct-cut in tower	Direct-cut in bunker
Dry matter	86.3	77.9	85.2	92.1	87.0	90.3
Crude protein	96.7	77.4	89.9	92.2	81.1	89.3
Ether extract	91.7	80.6	96.3	125.4	92.1	101.2
Crude fiber	98.2	93.0	98.7	100.7	96.0	97.2
N.F.E.	74.6	70.8	75.6	85.2	85.0	86.5
Ash	99.5	73.9	87.7	91.3	78.3	87.8
Sugar	8.2	23.5	30.1	29.3	37.1	42.1
Carotene	113.6	107.7	103.6	80.2	92.7	90.5

treatments was observed in Experiment 2. Better preservation of NFE and reducing sugars in Experiment 2 contributed noticeably to this difference.

The sources of dry matter loss by treatments are shown in Table 4. Top spoilage was negligible in all of the treatments, that occurring in the bunker silos being confined almost entirely to a narrow surface band next to the side walls. Seepage losses for the direct-cut tower-stored silages were four to five times greater than in the wilted silages and two to three times greater than in the bunker-stored silages. Differences between treatments with respect to gaseous losses were neither great nor consistent from year to year. However, gaseous losses for all treatments were much lower in Experiment 2 than in Experiment 1. The gaseous losses from plant respiration and/or through microbial activity were apparently less in Experiment 2. The lower losses of NFE and reducing sugars tend to support this observation, but the reasons for lower activity of this type are not apparent.

Average temperatures of Experiment 1, tower-stored silages, are presented in Table 5. The wilted silage reached a peak temperature of 80° F. four days after filling, after which the temperature declined until higher ambient temperatures were encountered in July. The temperature of direct-cut tower-stored silage increased to 79° F. during the ten days following filling, and remained rather constant until July. The relatively low temperatures of both silages indicated that air was very effectively excluded. The good-quality silage which resulted demonstrates that the development of higher temperatures is not necessarily a requirement of good ensiling techniques.

The temperatures of Experiment 2 silages also are shown in Table 5. Temperatures in the tower silos were higher than had been observed in the previous year.

TABLE 4
Distribution of dry matter losses (per cent of stored dry matter)

Source of loss	1956 Crop treatments			1957 Crop treatments		
	Wilted in tower	Direct-cut in tower	Direct-cut in bunker	Wilted in tower	Direct-cut in tower	Direct-cut in bunker
Spoilage	0	0	0.4	0	0	2.5
Seepage	1.7	8.2	3.7	1.4	6.6	1.8
Gaseous	12.0	13.9	10.7	6.5	6.4	5.4
Total	13.7	22.1	14.8	7.9	13.0	9.7

TABLE 5
Average silage temperatures, ° F.

	Experiment 1[a]			Experiment 2[b]			
		Silage treatment			Silage treatment		
Date	Days in storage	Wilted tower-stored	Direct-cut tower-stored	Date	Days in storage	Wilted tower-stored	Direct-cut tower-stored	Direct-cut bunker-stored
May 12	1	74	71	May 11	1	95	82	96
13	2	76	71	13	3	96	86	96
15	4	80	72	16	6	97	89	95
17	6	80	73	17	7	97	89	95
19	8	78	77	20	10	97	87	93
21	10	77	79	23	13	96	86	91
26	15	76	79	27	17	95	83	89
31	20	74	80	31	21	92	83	88
June 5	25	74	79	June 12	33	87	81	83
10	30	73	79	21	42	85	81	82
20	40	71	80	28	49	86	82	82
30	50	71	81	July 9	60	83	82	80
July 10	60	73	83	12	63	82	82	84
20	70	75	85	19	70	81	82	79

[a] Mean values for ½, 6½, and 12½ ft. above silo floor.
[b] Mean values for 3, 9, and 15 ft. above tower silo floors and mean values for all points in bunker silo.

Average maximum air temperatures during the filling periods were 72 and 77° F. for the first and second experiments, respectively, which could account for some of the differences in silage temperatures. Wilted silage and direct-cut tower-stored silage attained peak temperatures of 97 and 89° F., respectively, after six days of storage.

Temperatures at the several points in the bunker silo (Experiment 2) showed a range of 26° F. (81–107) one day after filling. These values gradually declined and became more uniform as the storage period progressed. Temperature values did not appear to be related to the depth of the silage, and the values in areas separated by only 1 ft. vertical distance differed by as much as 19° F. This seemingly random distribution may have been a reflection of differences in exposure to air during the filling process. Temperatures at a given location were rather uniform with respect to horizontal distribution. The average values for all points in the bunker silo on selected dates are presented in Table 5.

Results of the feeding and digestion trials are presented in Table 6. Comparisons of treatment effects within each year by analysis of variance indicated no significant differences in live weight gains or FCM production. The rates of FCM decline (regression) in Experiment 1 are deserving of special comment. Cows fed direct-cut tower-stored silage declined more rapidly in production during the preliminary periods than the two other groups. However, after their initial drop (2.26 lb. per cow per ten days) production of these animals increased somewhat during the remainder of the period (+0.96 lb. per cow per ten days). These circumstances resulted in similar average FCM production values for all treatments, in spite of the significantly different regressions during the experimental period (days 11–40).

TABLE 6

Results of feeding and digestibility trials

	Experiment 1			Experiment 2		
	Wilted tower-stored	Direct-cut tower-stored	Direct-cut bunker-stored	Wilted tower-stored	Direct-cut tower-stored	Direct-cut bunker-stored
Feed dry matter consumed *(pounds per cow per day)*						
Silage	23.34[c, d]	20.47[c]	20.06[d]	22.68	22.39	22.45
Concentrates	5.71	5.76	5.70	5.79	5.77	5.75
Total	29.05	26.23	25.76	28.47	28.16	28.10
(Pounds per 100 lb. live weight per day)						
Silage	2.34	2.09	2.07	2.05	2.04	2.07
Concentrates	0.57	0.59	0.59	0.53	.53	0.53
Total	2.91	2.67	2.66	2.57	2.56	2.59
Milk production (FCM) *(pounds per cow)*						
Average per day	24.51	23.53	25.11	24.54	25.73	25.60
10-day regression[a] (lb.)	−.35	+.96[e]	−.55[e]	−.68	−1.12	−.91
10-day regression[b] (lb.)	−1.47	−2.26	−1.09	−1.31	−.88	−.24
Ratio, Grain : Milk	1 : 4.3	1 : 4.2	1 : 4.4	1 : 4.2	1 : 4.5	1 : 4.5
Live weight per cow *(lb.)*						
Average	998	982	967	1,108	1,098	1,085
10-day regression[a]	+15.4	+15.2	+9.7	+11.2	+7.1	+13.1
Calculated feeding value						
Total daily requirement *(lb. TDN)*	21.18	21.70	19.16	20.50	19.34	21.31
Concentrate value *(lb. TDN)*	4.80	4.84	4.79	4.86	4.85	4.83
Required from silage *(lb. TDN)*	16.38	16.86	14.37	15.64	14.49	16.48
TDN content of silage, dry matter *(%)*	70.2	82.4	71.6	69.0	64.7	73.4
Determined feeding values of silages						
TDN in dry matter *(%)*	67.1	70.3	68.8	69.2	68.4	66.9
Dry matter digestibility (%)	69.0	70.9	71.6	69.6	70.1	69.0
Energy digestibility *(%)*				67.9	68.4	67.5
Feeding potential						
Feedable TDN/day *(lb.)*	15.66	14.39	13.80	15.69	15.31	15.02
Relative	113	104	100	104	102	100

[a] Regression by ten-day periods ending 20, 30, and 40 days after ration changes.

[b] Regression by ten-day periods ending ten and 20 days after ration changes.

[c, d, e] Difference in values followed by the same letter are statistically significant at the 1% level of probability.

Consumption of silage dry matter was significantly higher from wilted silage than from the two other silages in Experiment 1. However, dry matter intakes from the three silages in Experiment 2 were nearly identical.

Feeding values as determined by total collection digestion trials are presented in Table 6. All of the TDN values are above Morrison's (7) average value for orchardgrass silage, and there were no statistically significant differences among silages in either experiment. However, TDN values, calculated from estimated animal requirements, indicated that the silages varied widely in TDN content. This discrepancy between determined and calculated values indicates that ad lib. feeding will provide an expression of differences in silage dry matter intake, but is not well suited for TDN evaluations. When energy intakes are above requirements for milk production and body maintenance, the apparent energy value of a feed is profoundly affected by the relative efficiency of animals in converting feed energy to body energy and by the precision with which these changes can be measured. The efficiency of the animal also may be affected by level of feeding. Thus, silage energy evaluations by feeding trials with milking cows are at best rather crude and should be conducted on an equal dry matter intake basis over longer periods, in order to reduce these errors.

An ideal expression of feeding value should indicate the rate at which a feed will be consumed, as well as its nutritive value per unit of weight. Such an expression (pounds of silage dry matter consumed times determined per cent TDN) may be calculated from these data. This expression of feeding potential is presented in Table 6 as pounds of TDN per day and in relative terms. The relative rates of dry matter consumption are applicable only to the silages within an experiment which were directly compared under the same feeding condition; therefore, the feeding potential values are limited in the same way. The feeding potential of the Experiment 1 wilted silage was appreciably higher than the two other silages of that experiment. Other differences in feeding potential do not appear to be important.

REFERENCES

(1) Bratzler, J. W., Cowan, R. L., and Swift, R. W. Grass Silage Preservation with Sodium Metabisulfite. *J. Animal Sci.*, **15**: 163. 1955.

(2) Cowan, R. L., Bratzler, J. W., Keck, E., Jr., Swift, R. W., Alderman, G., and Washko, J. B. Further Experiments with Sodium Bisulfite as a Preservative for Grass Silage. *J. Animal Sci.*, **15**: 1188. 1956.

(3) Cowan, R. L., Keck, E., Jr., and Swift, R. W. Observation on Dry Matter Losses from a Covered and Uncovered Trench Silo. Pennsylvania State Agr. Expt. Sta., *Prog. Rept.* 150. 1956.

(4) Gordon, C. H., Melin, C. G., Wiseman, H. G., Irvin, H. M., and McCalmont, J. R. Chemical Quality, Nutrient Preservation, and Feeding Value of Silages Stored in Bunker Silos. *J. Dairy Sci.*, **41**: 1738. 1958.

(5) Gordon, C. H., Wiseman, H. G., Campbell, L. E., Melin, C. G., and Irvin, H. M. The Use of Acidifying Salts in High Moisture Hay-Crop Silages. *J. Dairy Sci.*, **37**: 659. 1954.

(6) LUCAS, H. L. A Method of Equalized Feeding for Studies with Dairy Cows. *J. Dairy Sci.*, **26**: 1011. 1943.

(7) MORRISON, FRANK B. *Feeds and Feeding.* 22nd ed. The Morrison Publ. Company, Ithaca, New York. 1957.

(8) NEWLANDER, J. A., AND RIDDELL, W. H. High-Moisture versus Wilted Grass Silage for Raising Dairy Calves. Vermont Agr. Expt. Sta., *Bull.* 602. June, 1957.

(9) ROGERS, CHARLES F., AND BELL, D. S. Acceptability of High-Dry Matter Silages. Ohio Agr. Expt. Sta., *Research Circ.* 20. June, 1953.

(10) SHEPHERD, J. B., WISEMAN, H. G., ELY, R. E., MELIN, C. G., SWEETMAN, W. J., GORDON, C. H., SCHOENLEBER, L. G., WAGNER, R. E., CAMPBELL, L. E., ROANE, G. D., AND HOSTERMAN, W. H. Experiments in Harvesting and Preserving Alfalfa for Dairy Cattle Feed. USDA, *Tech. Bull.* No. 1079. February, 1954.

(11) WOODWARD, T. E., AND SHEPHERD, J. B. Methods of Making Silage from Grasses and Legumes. USDA, *Tech. Bull.* 611. 1938.

EFFECT OF AUREOMYCIN, ERYTHROMYCIN, AND HYGROMYCIN ON THE GROWTH RATE AND WELL-BEING OF YOUNG DAIRY CALVES[1]

C. A. LASSITER, L. D. BROWN, AND C. W. DUNCAN
Departments of Dairy and of Agricultural Chemistry, Michigan State University, East Lansing

SUMMARY

Eighty-eight two-day-old calves were used in two 84-day experiments to evaluate the antibiotics aureomycin, erythromycin, and hygromycin as supplements to a milk replacer–starter type of ration. Hygromycin was fed at five levels ranging from 3.0 to 50 mg. of hygromycin per pound of replacer. A milk replacer–calf starter–hay system of feeding was used in both experiments.

Supplementing the rations with either aureomycin or erythromycin improved the rate of gain of the calves, increased the feed consumption, and reduced the incidence of calf scours. Hygromycin did not improve weight gains or skeletal growth of the calves, but did significantly reduce the incidence of calf scours. All levels of hygromycin tended to reduce the consumption of calf starter, particularly when fed at high levels.

The influence of several antibiotics, including aureomycin, on the growth and well-being of young dairy calves has been established and reviewed by Lassiter (1), but the value of feeding calves erythromycin and hygromycin has not been reported.

The purpose of this study was to evaluate the antibiotics erythromycin and hygromycin in comparison to aureomycin as constituents of calf feeds.

The results reported cover two separate experiments and will be designated as Trials I and II.

EXPERIMENTAL PROCEDURE

Trial I. Two-day-old Holstein calves were divided into five comparable groups and raised to 86 days of age on a milk replacer–starter–hay feeding program. The calves were permitted to remain with their dams for approximately 48 hr. after birth. Each calf was then placed in an individual pen, weighed, and subjected to one of five experimental regimes. The composition of the basal milk replacer follows: dried skimmilk—52.4 lb., cerelose—30 lb., corn distillers dried solubles—10 lb., dried whey—5 lb., dicalcium phosphate—2 lb., trace mineral salt—0.5 lb., and vitamin and mineral mix—0.1 lb. (3). All calves were fed the basal calf starter beginning at 1 wk. of age, composed of ground yellow corn—549 lb., crimped oats—470 lb., linseed oil meal—100 lb., soybean oil meal—100 lb., corn distillers dried solubles—150 lb., cane molasses—100 lb., dicalcium phosphate—15 lb., trace mineral salt—15 lb., and vitamin A and D supplement—1 lb. The basal milk replacer contained approximately 22% crude protein and the calf starter 16% crude protein.

Group 1 received the basal milk replacer and the basal calf starter, but no antibiotic supplement; Group 2 received the basal milk replacer supplemented with 50 mg. of aureomycin per pound and the basal calf starter supplemented

Received for publication May 7, 1959.

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 2436.

with 15 mg. of aureomycin per pound; Group 3 received the basal milk replacer supplemented with 50 mg. of erythromycin per pound and the basal calf starter supplemented with 15 mg. per pound of erythromycin; Group 4 received the basal milk replacer supplemented with 50 mg. of hygromycin per pound and the basal calf starter supplemented with 15 mg. of hygromycin per pound; and Group 5 received the basal milk replacer supplemented with 25 mg. of hygromycin per pound and the basal calf starter supplemented with 7.5 mg. of hygromycin per pound. All calves were fed a good quality alfalfa–brome grass hay, beginning at 1 wk. of age. All milk replacers were fed according to a prearranged schedule (Table 1) which was designed to have the calves completely

TABLE 1
Milk replacer feeding schedule

Age	Milk	Milk replacer	Water
(days)		*(lb/day)*	
0 – 2	Dam		
3 – 10	4.0	0.6	4.0
11 – 14	2.0	1.0	7.0
15 – 28	....	1.4	12.0
29 – 75	....	1.0	12.0
36 – 42	....	0.6	12.0
43 – 49	....	0.4	12.0
50 – over	....	None	

off the replacer feeding at 50 days of age. All starter and hay feeding was completely ad libitum, with the exception that starter consumption was limited to 5 lb. per calf daily. Complete records were kept on total feed intake, growth, scouring, and general health of the calves. The calves were weighed weekly and measurements of the height at withers and chest circumference were made at the beginning and end of the experimental period.

RESULTS AND DISCUSSION

Results of Trial I. The effect of the three antibiotics on the daily gain of the calves is shown in Table 2, effect on skeletal growth and incidence of scours in Table 3, and on feed consumption and feed efficiency in Table 4.

TABLE 2
Effect of aureomycin, erythromycin, and two levels of hygromycin on the average daily gain of Holstein calves (Experiment I)

Group	Initial Weight	Age in weeks		
		0–4	0–7	0–12
	(lb.)		*(lb/day)*	
1—Basal	97.6	0.43	0.65	1.04
2—Basal plus aureomycin	97.5	0.49	0.84	1.21
3—Basal plus erythromycin	96.6	0.44	0.68	1.12
4—Basal plus hygromycin (50 mg/lb replacer)	97.8	0.28	0.61	0.96
5—Basal plus hygromycin (25 mg/lb replacer)	97.9	0.35	0.71	1.01
L.S.D.—1%	n.s.	n.s.	n.s.	0.12
L.S.D.—5%	n.s.	n.s.	n.s.	0.09

n.s. = Nonsignificant.

TABLE 3

Effect of various antibiotics on skeletal growth and incidence of scours in calves from birth to 12 wk. of age (Experiment I)

Group	Average increase in		Scours
	Ht. of withers	Chest circumference	
	(in.)	*(in.)*	*(days/calf)*
1—Basal	4.62	7.00	11.0
2—Basal plus aureomycin	5.09	8.62	4.2
3—Basal plus erythromycin	4.84	8.31	3.1
4—Basal plus hygromycin (50 mg/lb replacer)	3.34	6.34	3.1
5—Basal plus hygromycin (25 mg/lb replacer)	4.59	6.84	4.2
L.S.D.—1%	0.54	n.s.	2.45
L.S.D.—5%	0.41	0.24	

n.s. = Nonsignificant.

The average initial weight of the five experimental groups was essentially the same; therefore, it should not have had any influence on the performances of the various rations (Table 2). The influence of the various antibiotics on the average daily gain of the calves at different ages is shown in Table 2. Aureomycin appeared to improve the growth rate of the calves more than did the two other antibiotics. The growth rate of the calves fed the rations containing aureomycin and erythromycin was significantly greater (2) than the growth rate of the calves fed the control ration or any of the rations containing hygromycin. Under the conditions of this experiment, and the level of supplementation employed, hygromycin did not appear to improve the growth rate of the calves. There was some indication that the growth rate of Group 5 calves was improved over that of the calves of Group 4, which might indicate that the level of supplementation with hygromycin was too high. This appears to be particularly evident as shown by the growth rate of the calves from 0 to 4 wk. of age. Both groups of hygromycin-fed calves exhibited a slower rate of growth during the first 4 wk. of life than any of the other antibiotic groups and the control calves.

TABLE 4

Effect of various antibiotics on average total feed consumption and feed efficiency of calves from birth to 12 wk. of age (Experiment I)

Group	Average total consumption				Feed/lb of gain
	Milk	Replacer	Starter	Hay	
	(lb.)				
1—Basal	40.0	42.4	188.1	43.8	3.2
2—Basal plus aureomycin	40.0	42.4	220.4	45.0	3.1
3—Basal plus erythromycin	40.0	42.4	201.7	34.8	3.0
4—Basal plus hygromycin (50 mg/lb replacer)	40.0	42.4	158.5	42.4	3.1
5—Basal plus hygromycin (25 mg/lb replacer)	40.0	42.4	162.7	53.8	3.1
L.S.D.—1%			n.s.	n.s.	n.s.
L.S.D.—5%			19.3	n.s.	n.s.

n.s. = Nonsignificant.

Skeletal growth data as shown in Table 3 show a trend similar to those of weight gains.

Under the conditions of this study, all of the antibiotics tested appeared definitely to decrease the incidence of scours or diarrhea, and all of the antibiotics appeared to be equally effective in this respect. As shown in Table 4, calves fed the ration containing aureomycin had the greatest consumption of starter and total feed. It appears also that hygromycin at both levels had a depressing effect upon the consumption of calf starter, but did not appear to have any appreciable effect on the consumption of hay. There were no significant differences among the various rations in respect to feed utilization; however, all of the antibiotics tended to increase the efficiency of utilization, but the calves fed the ration containing erythromycin had the highest efficiency.

Trial II. Forty-eight, two-day-old Holstein male and female calves were divided into six similar groups, based on sex and body weight. Group 1 received only a basal milk replacer and calf starter; Group 2 received the basal milk replacer supplemented with 50 mg. of aureomycin per pound and the basal calf starter supplemented with 15 mg. of aureomycin per pound; Groups 3, 4, 5, and 6 received the basal milk replacer supplemented with 3.0, 6.0, 12.0, and 25 mg., respectively, of hygromycin per pound and the basal calf starter supplemented with 1.0, 2.0, 4.0, and 7.5 mg., respectively, of hygromycin per pound. The basal milk replacer was composed of dried skimmilk—34.0 lb., dried whey—37.0 lb., cerelose—16.4 lb., corn distillers dried solubles—10.0 lb., trace mineral salt—0.5 lb., dicalcium phosphate—2.0 lb., and a vitamin and mineral mix—0.1 lb. The same calf starter was fed in this trial as was fed in Trial I. The basal milk replacer contained 20.7% crude protein. The feeding and management of the calves were essentially the same as outlined in Trial I.

Results of Trial II. As shown in Table 5, there were no significant differences among groups regardless of treatment, even though the differences in starting body weight were corrected by covariance analysis (2). Aureomycin-fed calves made slightly faster gains than the other groups up to 12 wk. of age and all groups of calves fed hygromycin made slightly slower gains than calves fed the basal ration. There appeared to be very little difference in the weight gains of

TABLE 5

Effect of aureomycin and four levels of hygromycin on the average daily gain of Holstein calves (Experiment II)[a]

Group	Average initial weight	Age in weeks	
		0–6	0–12
	(lb.)		
1—Basal	95.2	0.61	1.00
2—Basal plus aureomycin	99.8	0.62	1.08
Basal plus four levels of hygromycin			
3—(3 mg/lb replacer—1 mg/lb starter)	100.4	0.48	0.89
4—(6 mg/lb replacer—2 mg/lb starter)	93.0	0.48	0.89
5—(12 mg/lb replacer—4 mg/lb starter)	91.4	0.62	0.91
6—(25 mg/lb replacer—7.5 mg/lb starter)	92.9	0.49	0.89

[a] No significant differences among groups.

calves fed the four levels of hygromycin. In Trial I, both groups of calves fed hygromycin made weight gains slightly less than control calves. It would appear that the reduced rate of growth of calves fed hygromycin in Trial I was not due to an excessively high level of hygromycin.

There were no significant differences among groups in respect to increase in height at withers or chest circumference. None of the groups of calves had a very high rate of scouring and there was little evidence to indicate that either antibiotic had any significant effect. The calves averaged only 1.0, 0.6, 0.9, 1.0, 0.2, and 0.1 days per calf for Groups 1 to 6, respectively. The small differences among groups were not statistically significant.

The effect of the two antibiotics on feed consumption and feed required per pound of gain is presented in Table 6. The calves fed the ration containing aureomycin consumed slightly more starter than the control calves, or the calves fed any of the four levels of hygromycin, but the increase in starter consumption was not statistically significant. The calves fed the higher levels of hygromycin consumed less starter than any of the calves in the other groups, which confirms

TABLE 6

Effect of aureomycin and four levels of hygromycin on average total food consumption and efficiency of calves from birth to 12 wk. of age (Experiment II)

Group	Average total consumption				Feed/lb of gain
	Milk	Starter	Replacer	Hay	
	(lb.)				
1—Basal	40	42.4	218.1	69.3	4.1
2—Basal plus aureomycin	40	42.4	237.1	73.9	4.0
Basal plus four levels of hygromycin					
3—(3 mg/lb replacer—1 mg/lb starter)	40	42.4	215.2	79.0	4.7
4—(6 mg/lb replacer—2 mg/lb starter)	40	42.4	219.5	63.2	4.4
5—(12 mg/lb replacer—4 mg/lb starter)	40	42.4	212.1	75.2	4.4
6—(25 mg/lb replacer—7.5 mg/lb starter)	40	42.4	188.3	68.2	4.8

the observations made in Trial I. Very little difference existed among groups in hay consumption or feed required per pound gain.

The results of the two trials indicate that aureomycin and erythromycin improved the rate of gain in body weight of young dairy calves, reduced the incidence of calf scours, and increased the consumption of feed, particularly grain. The antibiotic hygromycin, at the levels fed in these studies, did not increase the rate of gain or feed consumption. In fact, at high levels, hygromycin depressed starter consumption, but it did appear to reduce the incidence of calf scours.

ACKNOWLEDGMENTS

This study was supported in part with a grant-in-aid from Eli Lilly and Co., Indianapolis, Ind., who also supplied the erythromycin and hygromycin. American Cyanamid Co., Pearl River, N. Y., supplied the aureomycin supplements; Chas. Pfizer and Co., Terre Haute, Ind., the vitamin A and D supplement; Midwest Dried Milk Co., Dundee, Ill., the dried whey; and Distillers Research Council, Cincinnati, Ohio, the corn distillers dried solubles.

REFERENCES

(1) LASSITER, C. A.. Antibiotics as Growth Stimulants for Dairy Cattle: A Review. *J. Dairy Sci.*, **38**: 1102. 1955.

(2) NOLLER, C. H., WARD, G. M., MCGILLIARD, A. D., HUFFMAN, C. F., AND DUNCAN, C. W. The Effect of Age of the Calf on the Availability of Nutrients in Vegetable Milk-Replacer Rations. *J. Dairy Sci.*, **39**: 1288. 1956.

(3) SNEDECOR, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames, Iowa. 1946.

UNUSUAL MAGNITUDE AND VARIATION OF PLASMA PROTEIN-BOUND IODINE VALUES OF DAIRY CATTLE[1,2]

R. O. ASPLUND,[3] G. A. McLAREN, H. O. HENDERSON, AND I. D. PORTERFIELD
Departments of Agricultural Biochemistry and Nutrition and of Dairy Husbandry,
West Virginia University, Morgantown

SUMMARY

Plasma PBI values of unusual magnitude and variations have been observed in the dairy herd at West Virginia University. The appearance of high PBI values is seasonal, occurring mostly in the late winter and spring. Plasma PBI follows a definite pattern with time by rising to a peak and then declining. A study of management practices revealed that the rise in PBI paralleled the feeding of grass silage to the dairy herd. High PBI levels were not associated with the feeding of excessive quantities of iodine compounds and were not characterized by high levels of plasma inorganic iodine. Fractionation revealed that, whereas some of the PBI was hormonally inactive, the remainder still indicated an unusually high level of circulating thyroid hormone. However, no symptoms of a pathological hyperthyroid condition could be observed in any of the animals studied. Although grass silage was not the sole cause of the elevation of PBI levels, it was capable of maintaining such levels when, normally, other conditions resulted in their decline.

The occurrence in cattle of either serum or plasma protein-bound iodine (PBI) levels which are considerably above what is generally regarded as the normal has not often been reported. Long *et al.* (7) found PBI values which they characterized as being ten to 20 times normal in cows fed iodinated casein. When the feeding of iodinated casein was discontinued, the PBI levels dropped rapidly but remained above normal for some time. Long, Gilmore, and Hibbs (6) reported PBI values of as high as 70 μg. % as a result of feeding large quantities of potassium iodide to cows. Mixner and Lennon (8) reported increases in plasma PBI levels to as high as 23 μg.% as a result of treating young bulls with thyroid hormones or thyroid-stimulating hormone. In each case, the elevated level of PBI was associated with the intake of large quantities of an iodine compound or with treatment of a thyroid-active substance.

The work reported in this paper was conducted to evaluate the use of plasma PBI as a means of predicting productive potential of dairy cattle. A preliminary phase of the work involved determining whether the variation between animals was significantly greater than the daily and seasonal variation. In the course of this statistical phase, exceptionally high PBI values were frequently obtained. These high values were apparently not related to a high iodine intake nor to any known substances possessing thyroid activity.

Received for publication May 26, 1959.

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 607. The material presented in this paper was taken from a dissertation submitted by R. O. Asplund to the graduate faculty of West Virginia University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Agricultural Biochemistry.

[2] This work was partially supported by funds from Northeastern Regional Project 30, Dairy Cattle Physiology.

[3] Present address: Department of Chemistry, University of Wyoming, Laramie.

EXPERIMENTAL PROCEDURE

The study of plasma PBI levels was carried out principally with heifer calves and cows of the Reymann Memorial Ayrshire herd of West Virginia University. The nature of the results was confirmed in the Holstein-Friesian and Jersey herds at the same farm. Blood samples were collected from each heifer calf at two-day intervals for either an eight- or 12-day collection period when the animals were 3 and 6 mo. of age. One or two blood samples were also collected from each dam. The blood samples were assayed for plasma PBI. The data obtained from calves at 3 and 6 mo. of age were analyzed for variance according to Snedecor (9).

The milking cows were fed a good-quality mixed hay consisting mostly of orchard grass, Ladino clover, and alfalfa, and either corn or hay-crop silage. They were fed as much roughage as they would consume. They were fed a grain mixture according to production, consisting of 300 lb. wheat bran, 300 lb. corn meal, 500 lb. ground oats, 100 lb. soybean oil meal, 18 lb. salt containing trace minerals, and 12 lb. bone meal.

The calves and heifers were fed a good-quality grass hay consisting mostly of orchard grass and timothy and corn or hay-crop silage. They were fed a grain mixture consisting of 300 lb. each of ground oats, wheat bran, corn meal, and soybean oil meal, together with 18 lb. salt containing some trace minerals and 12 lb. bone meal.

The hay-crop silage of special interest in this study was made in June each year. The forage used came from two fields; one of which consisted of wheat and vetch, and the other of orchard grass, Ladino clover, and alfalfa. The forage from these two fields was mixed in about equal quantities, with wheat and orchardgrass being the predominating grasses. Eight pounds of sodium metabisulfite was added to each ton of silage as a preservative.

The method employed for the determination of plasma PBI was that of Brown, Reingold, and Samson (4), as modified by Lennon and Mixner (5).

Chromatographic separation of the iodine compound bound to the protein of the plasma was accomplished by means of a combination of the methods of Acland (1) and Barna *et al.* (3). The iodine compounds were liberated from the precipitated protein by digestion with barium hydroxide and subsequent extraction with *n*-butanol. The butanol solution was evaporated to a small volume and divided into two equal parts. Carrier quantities of diiodotyrosine and thyroxine were added to one portion and the two parts were spotted in the same manner on different paper strips. The strips were developed with butanol–acetic acid solvent (4:1), dried, and the chromatograph containing the carrier compounds developed with ninhydrin. The corresponding spots on the other chromatograph were cut out and analyzed for iodine content by the same method used for the determination of PBI. Iodine recovered from the spot corresponding to diiodotyrosine was designated as the *D* fraction, and that recovered from the spot corresponding to thyroxine was designated the *T* fraction.

Iodine in whole plasma and in the feedstuffs was determined by the same method used for PBI. It has been pointed out by Acland (1), and confirmed by our experience, that in the use of this procedure iodine not bound to protein

is incompletely recovered. In our work, the recovery of iodine not bound to protein was 75%. In the analyses of iodine not bound to protein, a 75% recovery was assumed and an appropriate correction made.

RESULTS AND DISCUSSION

The mean plasma PBI values of Ayrshire cattle for the specified period of collection are shown in Table 1. Abnormally high PBI values occurred in late winter and spring each year and were also found in the cows during November-December, 1956.

TABLE 1

Mean PBI values of all blood samples collected from Ayrshire females

	PBI (μg. %) for specified period						
Age	Feb.-Mar. 1956	May 1956	Nov.-Dec. 1956	Feb.-Mar. 1957	June 1957	Nov.-Dec. 1957	Feb-Mar. 1958
3 mo.	4.5(20)	.....	7.1(52)	20.4(24)	.....	6.2(24)	19.4(12)
6 mo.	5.5(40)	12.8(16)	.....	25.1(40)	4.6(24)	.....	29.8(24)
Cows	.....	23.1(10)	26.7(12)	21.3(12)	3.2(5)	3.9(6)	25.4(9)

( )—Number of samples.

Between-animal variation was much greater in the calves than in the cows, even though the means are nearly the same. It was noted that 16.6% of the observed PBI values regarded as high in the 3-mo.-old calves were greater than 45.0 μg. %, whereas the corresponding figure for the 6-mo. age groups was only 9.7%. Values of such magnitude were never encountered in cows. The summaries of the analysis of variance for PBI of 3- and 6-mo.-old Ayrshire females are shown in Table 2. It is apparent that the results are quite similar at both ages. Therefore, the relationship of PBI values found in calves and those found in cows eliminates their possible use in predicting the productive potential.

Spot checks of the Holstein-Friesian and Jersey herds indicated quite clearly that the same situation found at a given time in the Ayrshire herd also existed in these herds. It is interesting that at no time could any gross symptoms of hyperthyroidism be detected in any of these animals, despite the fact that values regarded as being from three to ten times above normal were regularly encountered. Age at weaning, onset of puberty, and amount of exposure to sunlight could not be found to have any relationship to plasma PBI levels.

TABLE 2

Summary of analysis of variance of plasma PBI data from 3- and 6-mo.-old Ayrshire females

	Three-month-old		Six-month-old	
Source of variation	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Periods	4	1,015.8**	4	5,246.7**
Individuals within periods	28	474.8**	36	307.4*
Plasma samples	99	12.2	103	16.2
Total	131		143	

* $P < .05$.
** $P < .01$.

During periods when values were elevated, this elevation in PBI values could be shown to have a regular pattern. In Figure 1 are represented the combined daily means of plasma PBI determinations from 3- and 6-mo.-old heifers sampled in February and March, 1957 and 1958. In both years, the values reached a peak in March and then declined. An important difference between the two curves is that in 1957 the same animals were not necessarily sampled on a given day, whereas in 1958 the same group of animals was sampled continuously through the collection period.

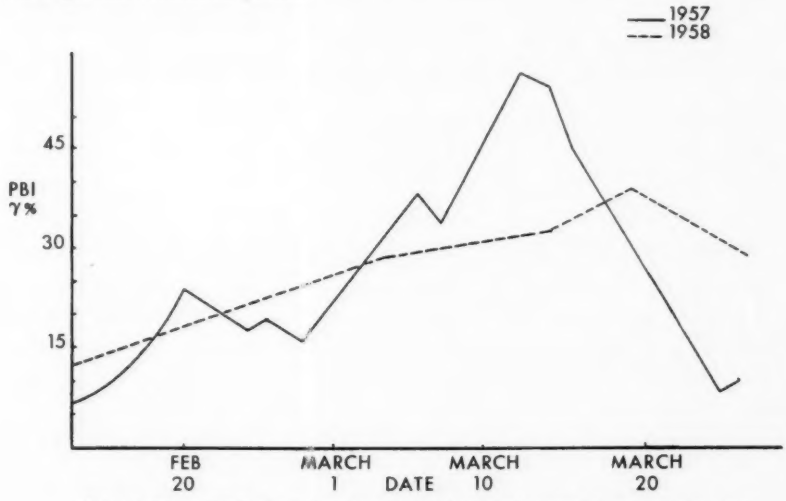


FIG. 1. Mean PBI of all female calves (Ayrshire) sampled on a given date.

The possibility of an error in the PBI analytical method as used in this laboratory was considered. Plasma samples obtained from the West Virginia University Ayrshire herd were submitted to six laboratories for PBI analysis. In four of the laboratories, PBI was analyzed by means of the alkaline ashing procedure and in the other two laboratories the iodine distillation procedure was used. The plasma samples submitted to these laboratories contained both abnormally high and normal PBI values. The results obtained were not significantly different ($P < .05$) from those obtained in this laboratory. These data are presented in Table 3.

The iodine content of the ration ingredients received by these animals is shown in Table 4. None of the ingredients contained appreciable quantities of iodine. From Table 4, it can be calculated that a large dairy cow fed daily 15 lb. of hay, 30 lb. of corn silage (fresh weight), and 15 lb. of concentrate would ingest a daily quantity of only 0.2 mg. of iodine. Long, Gilmore, and Hibbs (6) fed as much as 22 mg. daily of potassium iodide to dairy cows without affecting PBI levels.

According to Long *et al.* (6, 7) and Allcroft *et al.* (2) elevated PBI levels resulting from feeding iodine compound are accompanied by extreme elevation of

TABLE 3
Plasma PBI values obtained in different laboratories using two methods of analysis

	Plasma PBI (μg. %) in sample							
	6[a]	47[b]	52A[b]	52B[b]	63B[b]	65[c]	74[c]	78[c]
A[d]	6.8	12.1	14.4	14.5	....	....	.....	.....
B[d]	7.6	12.3	19.2	21.4	....	....	.....	.....
C[d]	8.1	11.7	20.7	23.3	....	....	.....	.....
D[d]	6.9	10.6	15.8	18.4	....	....	.....	.....
H[e]	....	.....	.....	.....	6.5	7.6	.....	.....
p[e]	....	.....	.....	.....	....	....	17.0	82.7
WVU[d]	6.3	10.8	17.1	16.0	6.3	6.3	15.0	76.0
Mean and S.E.	7.1±0.3	11.5±0.3	17.4±1.2	18.7±1.7				

[a] This was a sample of pooled human plasma purchased from Warner-Chilcott Co., New York.
[b] Plasma obtained from an Ayrshire cow.
[c] Plasma obtained from an Ayrshire calf.
[d] Plasma PBI determined by means of alkaline ashing.
[e] Plasma PBI determined by means of iodine distillation.

TABLE 4
Iodine content of the ration received by the West Virginia University dairy herd

Component	Iodine (μg. per gram)
Concentrate fed to cows	0.008
Concentrate fed to calves	0.009
Hay (fed to milking herd)	0.005
Hay (fed to calves)	0.004
Corn silage (dried)	0.015
Grass silage (dried)	0.009

the level of iodine in plasma not bound to protein (plasma inorganic iodine). They reported that PBI levels of 30 to 70 μg. % are accompanied by inorganic iodine levels of from 400 to 1,100 μg. %. According to these workers, PBI would represent about 10% of the total blood iodine. It can be seen from Table 5 that with samples chosen at random from those collected in this study, in every case the PBI represented more than 90% of the total blood iodine.

The division of PBI into the *D* and *T* fractions is presumably a division into hormonally inactive and active fractions, respectively. Long, Gilmore, and Hibbs (6) have suggested that in the case of high PBI values in which no symp-

TABLE 5
A comparison of plasma PBI and total plasma iodine of Ayrshire females

PBI	Total iodine	PBI % of total iodine
(μg. %)	*(μg. %)*	
7.0	7.8	89.7
5.4	6.1	88.5
6.6	7.4	89.2
4.8	4.9	98.0
16.3	18.8	86.7
29.0	32.3	89.8
38.7	40.9	94.6
52.8	57.0	92.6
Mean	.....	91.1

toms of hyperthyroidism were present a large part of this PBI was represented by hormone-like iodination products of protein, such as the hormonally inactive products in iodinated casein. It would be expected that such products would be in the *D* fraction and active compounds would be in the *T* fraction. In Table 6 it may be seen that, in contrast to normal PBI levels, high PBI levels are characterized by a considerable percentage of iodine in the *D* fraction. However, the level in the *T* fraction is much higher than that regarded as normal and should reflect a pathological thyroid state which was not in evidence in this study.

TABLE 6

Chromatographic fractionation of PBI from cattle with high and normal levels. Values expressed in micrograms per 100 ml. of plasma

Sample type	*D* fraction	*T* fraction	PBI	Per cent recovery
High	14.5	21.1	44.0	81
High	16.0	28.8	60.1	75
Normal	0	3.8	4.5	84
Normal	0	3.8	4.1	93

The appearance of high plasma PBI values corresponded quite closely to the time at which grass silage feeding was begun. In the fall of 1956, the cows were fed all grass silage in contrast to the fall of 1957, when both corn and grass silage were fed. In the fall of 1956, one 9-mo.-old heifer which had received grass silage had a mean plasma PBI for four collections of 48.3, compared to a mean of 8.2 for four other animals of the same age which had not received silage. In the spring, the rise of the plasma PBI of calves corresponded approximately to the beginning of grass silage feeding. It would appear that while grass silage is not the sole cause of the elevation in PBI, it is a contributing factor of some importance.

REFERENCES

(1) Acland, D. J. The Estimation of Serum Protein-Bound Iodine by Alkaline Incineration. *Biochem. J.*, **66**: 177. 1957.

(2) Allcroft, R., Salt, F. J., and Hignett, S. L. The Effect of Commercial Phenothiazine on Blood Iodine Values in Sheep. *Vet. Record*, **67**: 626. 1955.

(3) Barna, S., Szabo, G., Fever, G., and Balagh, I. R. Determination of Thyroxine and Triiodothyronine in the Serum for Application in the Differential Diagnosis of Thyroid Disease. *Acta Med. Hung.*, **10**: 339. 1957.

(4) Brown, H., Reingold, A. M., and Samson, M. The Determination of Plasma Protein-Bound Iodine by Dry Ashing. *J. Clin. Endocrinol. Metabolism*, **13**: 444. 1953.

(5) Lennon, H. O., and Mixner, J. P. Some Factors Affecting the Determination of Plasma Protein-Bound Iodine Using the Alkaline Fusion-Ceric Sulfate Method. *J. Dairy Sci.*, **40**: 351. 1957.

(6) Long, J. F., Gilmore, L. O., and Hibbs, J. W. The Effect of Different Levels of Iodide Feeding on Serum Inorganic and Protein-Bound Iodine Levels. *J. Dairy Sci.*, **39**: 1323. 1956.

(7) Long, J. F., Gilmore, L. O., Hibbs, J. W., and Ely, F. The Effect of Thyroprotein Feeding on the Level of Protein-Bound Iodine and Inorganic Serum Iodine in the Bovine. *J. Dairy Sci.*, **35**: 503. 1952.

(8) Mixner, J. P., and Lennon, H. D. Effects of Thyrotrophin, L-Thyroxine, and L-Triiodothyronine on Levels of Plasma Protein-Bound Iodine in Dairy Bull Calves. *J. Dairy Sci.*, **41**: 840. 1958.

(9) Snedecor, G. W. *Statistical Methods.* Iowa State College Press, Ames. 1956.

TECHNICAL NOTE

EVALUATION OF SHORT-INTERVAL MILKING AS A PHYSIOLOGICAL TECHNIQUE

While attempting to evaluate short-interval milking as an experimental approach to problems in milk secretion in dairy cattle, Lakshmanan *et al.* (1) observed that certain factors appeared to influence the amount of milk and butterfat produced during two-hourly milking for a short period. Because of the possible importance of this technique, we should like to make the following comments on those aspects of the paper which we feel may lead to ambiguity in interpretation.

1. Production in any 24-hr. period can not be regarded as a valid response criterion; hence, the analyses of variance presented are based on "observations within plots" and are invalid. The three "experiments" were, in fact, three uniformity trials; the three observations on each cow provide only a single measure of the response to short-interval milking. Thus, two reasonable measures of response per cow would be,

$$R = \text{Period II} - \left(\frac{\text{Period I} + \text{Period III}}{2}\right)$$

or alternately,

$$R = \frac{2\ \text{Period II}}{\text{Period I} + \text{Period III}}$$

That the three observations provide only one piece of information is obvious when it is considered that if, for any cow, the Period II observations were lost, the remaining observations on that cow would be meaningless.

2. The general procedure in controlled experimentation is to apply treatments to experimental units and to measure the resulting yield. In Lakshmanan *et al.* (1), the experimental units were cows, the treatments: (a) high producers early in lactation; (b) low producers early in lactation; (c) high producers late in lactation; and the yield was response to two-hourly milking. With 13 cows, their data had a total of 12 degrees of freedom, for which the correct analysis of variance would be:

Source of variation	*DF*
Between treatments	2
Error (cows within treatments)	10
Total	12

This analysis, of course, would require all cows to be milked under the same conditions. In fact, however, treatment (b) was carried out at a different time than the other treatments; hence, its effect was confounded with the different shed conditions prevailing at the time. Nevertheless, accepting this restriction, it is clear that the authors could have carried out a valid statistical test of the hypothesis that the potential level of production per cow influenced the response to short-interval milking.

3. The simplest hypothesis on the response to frequent milking would probably be that at a particular stage in lactation each cow has a potential production, P, but that when milked, for example twice-daily, the cow produces an amount, T, less than or equal to P. Yield of potentially low-producing cows at all stages of lactation is unlikely to be influenced by frequent milking, hence,

$$T = P \qquad (1)$$

At higher levels of potential production, however, twice-daily milking might be expected to limit actual production to a fraction of potential production, that is,

when $T < P$,

$$T = a + bP \qquad (2)$$

where $0 < b < 1$.

Taking two-hourly milking for 24 hr. as a measure of potential production, P, and twice-daily production as a measure of T, the following estimate of Equation (2) was obtained from the present data by regression:

$$T = 7.99 + 0.7755\,P. \qquad (2')$$

This relation, which has a coefficient of determination, r^2, of .96 is shown graphically in Figure 1, where the maximum value of P equal to T is estimated to be 35.6 lb. of milk per 24 hr.

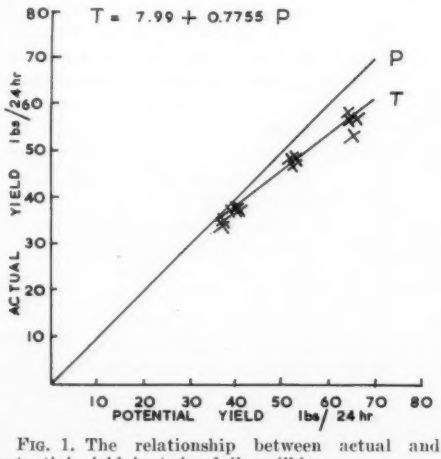


FIG. 1. The relationship between actual and potential yield in twice-daily milking.

In computing (2′), the data for the cow in its first month of lactation were omitted, because the relationship between actual and potential yield in early lactation is complicated by the unknown factors responsible for the positive slope of the lactation curve in early lactation.

It should be noted that the regression technique used to compute (2′) is open to criticism because of the identity,

$$P = T + \text{difference}.$$

Nevertheless, (2′) is probably the most illuminating use that can be made of the available data.

4. No justification is given for selecting a period in which the cows are taken to be adjusting to the new milking regimes. We may observe that since the mean yield for Period III was greater than for Period I, and since the cows were nearer to the end of their lactation in Period III, it is possible that an overcompensation by each cow took place. If shown to be true, a finding such as this would have great physiological significance. To test accurately for compensation, it is necessary to take into account the regression of time on yield (i.e., on the slope of the lactation curve). This might be accomplished through using a slope calculated from a large population of cows or, alternately, a more simple method might be a comparison of the slopes in the two periods.

D. R. Lamond
Faculty of Rural Science, and
W. V. Candler
Faculty of Agricultural Economics,
University of New England,
Armidale, N.S.W., Australia

REFERENCE

(1) Lakshmanan, S., Shaw, J. C., McDowell, R. E., Ellmore, M. F., and Fohrman, M. H. Short-Interval Milking as a Physiological Technique. I. Effect of Frequent Milking with the Aid of Oxytocin on Milk and Milk Fat Production. *J. Dairy Sci.*, **41**: 1601. 1958.

OUR INDUSTRY TODAY

DRY VS. LYE-SOLUTION STORAGE OF MILKING MACHINE RUBBER INFLATIONS[1]

J. M. JENSEN
Department of Dairy, Michigan State University, East Lansing

Milking machine rubber inflations deteriorate at an accelerated rate as a result of the absorption of fats during milking. This fat absorption and extraction by and from various natural and synthetic rubbers have been studied by several workers (1-5) who, in general, showed the damaging effects of fat on rubber and the benefits of lye storage.

Milking machine inflations undergo changes in size, shape, and composition as a result of use and storage during use interims. The rapidity with which these changes take place determines the useful life of inflations. If this period of usefulness could be prolonged by improved storage conditions, the savings effected thereby to the milk producers would be of incalculable value. To this end, a long-range, farm-use storage study was made with different brands of natural and Perbunan rubber inflations. The effects of dry vs. lye-solution storage on the deterioration and life of milking machine rubber inflations were compared.

HISTORICAL

Investigations dealing with the deterioration of rubber by absorption of fats and removal of fats with caustic soda, thereby extending its life, have been conducted by workers in New Zealand and England as well as in the United States (1-10). Fat absorption has been shown to take place largely during the time of milking, with approximately half of the fat absorbed from the milk and half from the skin and hair of the cows.

Deterioration of rubber occurs in two stages: namely, the primary, causing softening and swelling and the secondary, resulting in oxidation and hardening of the rubber. The primary types of deterioration are largely reversible, but the secondary ones are irreversible.

Laboratory studies, as well as field studies, show that fats are effectively and conveniently removed by storing inflations in 5–10% strength solutions of cold caustic soda for periods of 1-2 wk. With this procedure, Gardner and Berridge (4) noted a 50% increase in the useful life of inflations.

SCOPE OF THE INVESTIGATION

This study on dry vs. lye-solution storage of milking machine rubber inflations was made under actual field conditions. Both natural and synthetic rubber inflations were subjected to 6 mo. use for each of the two methods of storage. The year-long investigation, embracing all-season conditions, involved 36 milk producers, who used the rubber inflations according to specific directions. The study encompassed 360 natural and 232 synthetic rubber inflations, which were inspected intermittently throughout the year and checked carefully at the end of the use period.

EXPERIMENTAL PROCEDURE

Milk producers in Central Michigan were selected for the study according to routes. Producers were asked to use sets of milking-machine inflations for a week, then replace the inflations and store them either dry or in a 5% lye solution, as the experiment dictated. During use, inflations were stored dry between milkings. Producers washed their inflations without specific instruction. All inflations placed on all the milking machines were new and branded with their respective weights. Each producer was given two sets of inflations so they could be alternated at weekly intervals. Extra inflations, identified by special marking, were furnished for replacing damaged ones.

The inflations used were of standard, natural rubber and of Perbunan rubber quality as furnished by various manufacturers.

Equipment for lye storage consisted of containers, racks, and lye was provided. Earthenware crocks were used at the start of the study but were replaced later with 5-gal. steel pails.

Producers were requested to place tags on inflations which required removal from service, with notation of date of discontinuance. At the end of 1 yr., or 6 mo. use for each set of inflations, all the inflations were picked up for reweighing and laboratory examination.

RESULTS

Effects of dry storage. Data presented in Table 1 show the effects of storing inflations dry for seven-day periods after each week of use.

All the natural rubber inflations, regardless of brand, increased in weight. The increase in weight per brand of inflation ranged from 7.4–15.8%. The average increase in weight was 10.8 g. per inflation, or 10.4% of the original inflation weight.

All Perbunan inflations lost weight equal to 1.73% of the original weight. The percentage

[1] Authorized for publication as Journal Article No. 2414 of the Michigan Agricultural Experiment Station.

TABLE 1

Weight changes of natural and synthetic rubber inflations during 12 mo. of use when stored dry during alternate weeks

Brands of rubber used	Cows milked (No.)	Average initial weight of inflations (g.)	Change in weight of inflations (g.)	(%)
		Natural rubber		
B1	3,600	87.3	+7.2	8.2
B2	3,832	87.1	+10.4	11.9
F1	3,280	123.7	+9.2	7.4
F2	4,015	123.4	+14.4	11.7
F3	2,525	124.0	+10.4	8.4
A	2,525	92.1	+14.6	15.8
D	3,030	90.8	+7.4	8.1
		Average	10.8	10.4
		Perbunan rubber		
H1	2,555	87.4	−1.5	−1.7
H2	1,642	87.5	−0.8	−0.9
H3	3,650	87.3	−1.3	−1.5
H4	2,007	87.4	−1.1	−1.3
H5	3,650	86.6	−1.2	−1.4
H6	2,900	86.5	−1.5	−1.7
H7	3,100	88.0	−3.2	−3.6
		Average	−1.05	−1.73

loss of the inflations ranged from 0.9–3.6% per inflation.

Of 136 natural rubber inflations used 97, or 71.3%, were discarded during the period of study; whereas, only 16 of 120 Perbunan inflations, 13.3%, were discarded during a similar period (Table 2).

Effects of lye-solution storage. Data presented in Table 3 show the effects of 5% lye solution on weight changes of milker inflations used during the 12-mo. study.

The natural-rubber inflations gained on an average 3.94 g. per inflation, or 3.87%. Except for Brand D, which lost 2.1 g. per inflation, the gains ranged from 2.8–7.2 g.

The Perbunan inflations lost weight with lye storage; the average loss was 7.1 g. each, or 8.1%. The losses ranged from 6.7 to 9.5%.

Sixty-five of the 159 natural rubber inflations used and stored in lye, or 28.1%, were discarded as a result of damages sustained by them (Table 4). Damage consisted of roughness inside inflation barrels in 48 inflations, and from stem tears and punctures in 17 inflations.

Reasons for discarding inflations. Data presented in Table 5 show the nature and extent of damage to natural and Perbunan rubber inflations under dry and lye storage.

Of 136 natural-rubber inflations subjected to alternate-week dry storage 97, or 71.3%, were discarded. Forty-three inflations, 31.6% of the total number used, were discarded because of stem damage, and 54, or 40%, because of damage to inflation barrels. Thus, of 97 natural-rubber inflations discarded, 44% had stem damage and 56 had barrel damage.

Of 224 natural-rubber inflations subjected to lye storage 65, or 29%, were discarded. Only 8.5% of the total number used were discarded because of stem damage, and 20.5% because of barrel damage. Thirty per cent of those discarded were stem-damaged and 70% were barrel-damaged.

TABLE 2

Milking-machine rubber inflations discarded during 12 mo. of use when stored dry during alternate weeks

Brands of rubber used	Cows milked (No.)	Inflations			
		At start of study (No.)	At end of study (No.)	Discarded during study (No.)	(%)
			Natural rubber		
B1	3,600	24	0	24	100.0
B2	3,832	32	23	9	28.0
F1	3,280	16	0	16	100.0
F2	4,015	24	0	24	100.0
F3	2,525	8	8	0	0.0
A	2,525	8	8	0	0.0
D	3,030	24	0	24	100.0
		Total 136	39	97	71.3
			Perbunan rubber		
H1	2,555	16	15	1	6.3
H2	1,642	16	16	0	0.0
H3	3,650	16	12	4	25.0
H4	2,007	16	13	3	18.8
H5	3,650	24	20	4	16.7
H6	2,900	16	15	1	6.3
H7	3,100	16	13	3	18.8
		Total 120	104	16	13.3

TABLE 3
Weight changes of natural and synthetic rubber inflations during 12 mo. of use when stored alternate weeks in a 5% lye solution

Brands of rubber used	Cows milked (No.)	Average initial weight of inflations (g.)	Change in weight of inflations (g.)	(%)
Natural rubber				
A1	912	90.3	+5.1	5.6
A2	1,825	92.0	+4.4	4.8
A3	3,285	92.0	+7.2	7.8
B1	4,197	87.1	+3.1	3.6
B2	2,920	86.8	+2.8	3.2
C1	2,525	98.6	+3.5	3.5
C2	1,825	98.9	+4.9	4.9
D	1,825	91.1	−2.1	−2.3
E1	1,896	99.8	+4.8	4.8
E2	1,825	106.5	+6.0	5.6
F1	3,650	122.8	+3.1	2.5
F2	2,920	123.5	+3.5	2.8
F3	2,190	123.2	+5.4	4.4
G	1,581	113.7	+3.5	3.1
		Average	3.94	3.87
Perbunan rubber				
H1	912	97.2	−6.5	6.7
H2	1,460	88.4	−8.4	9.5
H3	1,825	87.9	−7.1	8.1
H4	2,723	87.1	−6.8	−7.8
H5	1,277	80.0	−6.5	−8.1
H6	2,190	86.9	−7.4	−8.5
H7	3,650	81.9	−7.0	−8.5
H8	3,467	86.8	−6.9	−7.9
		Average	−7.1	−8.1

Of 120 dry-stored Perbunan inflations 16, or 13.3%, were discarded because of stem damage. Of 112 lye-stored Perbunan inflations 12, or 10.07%, were discarded for the same reasons. No Perbunan inflations were discarded because of barrel damage.

Appearance of inflations at close of study. Natural rubber inflations differed widely in appearance after storage, depending upon the type of storage. The dry-stored inflations became longer, of greater diameter, and appeared distended and swollen. Furthermore, many inflations were misshapen, to the extent that when placed on end they did not stand upright (Figure 1).

On the other hand, those inflations stored in the 5% lye solution retained their original shape and size. All natural-rubber inflations, as a whole, were equally rough as determined by feel and appearance.

Both Perbunan and natural rubber inflations, after several weeks of dry storage, were coated with a grayish film of fat which had been expressed by the rubber. The heaviest coating of film appeared in the flexing portion of the inflation barrel. No such coating appeared on those inflations stored in lye solution. Lye-stored inflations retained a clean, fresh, new appearance throughout the study.

FIG. 1. Showing differences in appearance and size of inflations, following dry storage (left one of each pair) and lye storage.

Perbunan inflations retained their original shape, regardless of the type of storage. Indications of swelling or deterioration of the Perbunan were not noted. However, some roughness inside the barrels was observed. The lye-stored Perbunan inflations were damaged slightly more than were those stored dry. Also, the lye-stored inflations shrank in over-all size to a small extent. Presumably, the shrinkage was equal to the difference between the losses in weight when the inflations were stored under either of the two conditions. Therefore, the Perbunan inflations under lye storage were presumed to be approximately 6.5% smaller than those dry stored.

A further outstanding difference resulting from the type of storage was the odor of the inflations. Regardless of the type of inflation, dry storage yielded an unclean odor. This was in contrast to the very clean, fresh odor found in those inflations stored in lye solution.

Containers for lye storage. New, earthenware 5-gal. crocks were supplied producers using the lye-storage treatment of milking-machine rubber inflations. After about 2 mo. of use, lye solution seepage occurred, due to the lye destroying the glazed finish on the crocks. The crocks were then replaced with 5-gal., 24-gauge steel pails. The pails selected were fitted with gasketed covers which were closed by a level-locking ring. For convenience in handling, the pails were provided with bails.

The steel pails proved satisfactory for lye storage. They did not leak and they were not subject to lye corrosion. Some rust spots appeared on the underside of the cover, resulting from condensation of moisture. The steel pails were easy to handle and, being covered, gave protection against entry of soiling matter. The element of danger in the use of strong lye in an open container was avoided by using a locking cover. The kind of pail used is shown in Figure 2, along with an inner polyethylene pail to hold the inflations. The polyethylene pail was punched with holes, making it serve as a colander, for rapid entry and discharge of the lye solution.

TABLE 4

Milking machine rubber inflations discarded during 12 mo. of use when stored alternate weeks in a 5% lye solution

Brands of rubber used	Cows milked (No.)	Inflations			
		At start of study (No.)	At end of study (No.)	Discarded during study (No.)	Discarded during study (%)
Natural rubber					
A1	912	8	6	2	25.0
A2	1,825	16	15	1	6.3
A3	3,285	16	16	0	0.0
B1	4,197	16	14	2	12.5
B2	2,920	16	16	0	0.0
C1	2,525	16	8	8	50.0
C2	1,825	8	7	1	12.5
D	1,825	16	16	0	0.0
E1	1,896	24	0	24	100.0
E2	1,825	16	14	2	12.5
F1	3,650	16	12	4	25.0
F2	2,920	16	16	0	0.0
F3	2,190	24	19	5	20.8
G	1,581	16	0	16	100.0
	Total	224	159	65	29.0
Perbunan rubber					
H1	912	8	8	0	0.0
H2	1,460	16	13	3	18.8
H3	1,825	16	16	0	0.0
H4	2,723	16	14	2	12.5
H5	1,277	8	7	1	12.5
H6	2,190	16	12	4	25.0
H7	3,650	16	14	2	12.5
H8	3,467	16	16	0	0.0
	Total	112	100	12	10.7

TABLE 5

Nature of damage to natural and synthetic rubber inflations when subjected to dry vs. lye storage

Type of rubber	Kind of storage	Inflations			Inflations discarded because of					
		Used (No.)	Discarded		Stem damage			Barrel damage		
			(No.)	(%)	(No.)	(%)	(%)[a]	(No.)	(%)	(%)[a]
Natural	Dry	136	97	71.3	43	31.6	44.0	54	40.0	56.0
Natural	Lye	224	65	29.0	19	8.5	30.0	46	20.5	70.0
Perbunan	Dry	120	16	13.3	16	13.3	100.0	....	.....	.....
Perbunan	Lye	112	12	10.7	12	10.7	100.0	....	.....	.....

[a] Percentage of total discards.

DISCUSSION

The seven-day, alternate-week storage in 5% lye solution was effective in prolonging the useful life of inflations, the retention of their original shape and size, as well as in keeping them fresh in odor and clean in appearance. While natural rubber was benefited by lye storage more than was Perbunan, both kinds of inflations were made clean and fresh of odor to an extent that was never attained by the best washing practices.

An increase in weight of inflations was recognized as being caused by fat having been absorbed from the milk and from the skin of the cow's udder, the absorption taking place mainly during milking. That portion of the inflation subjected to flexing with the pulsation of the milker absorbed the greatest amount of fat. Apparently, once flexing was started in an area of an inflation, the pattern of flexation became established and continued there despite removal of inflations for cleaning after each milking. Absorption of fat occurred also in nonflexing portions of the inflations, as observed by films of fat which appeared on idle inflations after several weeks of storage.

FIG. 2. Showing use of lye storage containers.

Natural rubber inflations increased in weight with absorption of fat. Perbunan, on the other hand, showed loss in weight. A similar characteristic of Perbunan was shown in a previous investigation (5), wherein loss of weight was experienced when Perbunan was soaked in butter oil. Perbunan inflations, nevertheless, absorbed some fat as indicated by an appearance of fat films on inflations held in the laboratory several weeks at the completion of the study.

SUMMARY AND CONCLUSION

Natural and Perbunan rubber milking machine inflations were stored dry and in a 5%, cold-lye solution during alternate weeks under field conditions for a year to compare their lasting qualities, weight changes, and appearances.

With dry storage, the natural rubber inflations gained an average of 10.4% in weight. Similarly stored Perbunan inflations lost weight, averaging 1.73%.

When stored in the lye solution, the natural rubber inflations increased in weight, averaging 3.87%. Under similar conditions of storage, the Perbunan inflations lost weight, averaging 8.1%.

A higher percentage of natural rubber inflations, 71.3% as compared with 29%, was discarded when the inflations were stored dry rather than in lye solution. Perbunan inflations were equally durable under either condition of storage.

Lye-solution storage favored retention of shape and quality of the natural rubber inflations, whereas those dry-stored became enlarged and misshapen with use.

Lye-solution storage yielded cleaner appearing and sweeter-smelling inflations than dry storage, regardless of the type of inflation.

ACKNOWLEDGMENT

The author gratefully acknowledges financial support and assistance with the inflation materials used in this study by the United States Rubber Company, Passaic, New Jersey; also, to Ira Wilson & Sons Dairy Company, Detroit, for cooperation with producer sampling.

REFERENCES

(1) CLARK, M., BERRIDGE, N. J., AND GARDNER, E. R. The Determination of Milking Rubbers. V. The Effect of Rubber Composition. *J. Dairy Research*, 22: 144. 1955.

(2) COOPER, J. H. The Deterioration of Milking Rubbers. IV. Fat in Milk Liners. *J. Dairy Research*, 22: 138. 1955.

(3) COOPER, J. H., AND GARDNER, E. R. The Deterioration of Milking Rubbers. III. The Effect of Farm Treatment. *J. Dairy Research*, 20: 340. 1953.

(4) GARDNER, E. R., AND BERRIDGE, N. J. The Deterioration of Milking Rubbers. II. The Effect of Fat. *J. Dairy Research*, 19: 31. 1952.

(5) JENSEN, J. M. Fat Extraction from Milker Rubber with Lye Solutions. *J. Dairy Sci.*, 38: 835. 1955.

(6) MOIR, G. M. Milking Machine Rubberware. New Zealand Dept. Agr., *Bull.* 118. 1944.

(7) THIEL, C. C., CLOUGH, P. A., CLEGG, L. F. L., HIRONS, ELIZABETH, AKAM, D. N., AND GRUBER, MARIE. Immersion Cleaning of Milking Equipment. *J. Dairy Research*, 22: 156. 1955.

(8) THIEL, C. C., CLEGG, L. F. L., CLOUGH, P. A., AND COUSINS, CHRISTIANA M. Further Studies of Immersion Cleaning of Milking Equipment. *J. Dairy Research*, 23: 217. 1956.

(9) WARD, G. M., ELSIE, V. R., AND HURRY, J. A. The Effect of Milking Practices upon the Deterioration of Milking Machine Inflations. *J. Milk Food Technol.*, 20 (11): 312. 1957.

(10) WHITTLESTONE, W. G. The Cleaning of Milking Machine Rubberware. (Reprint.) *J. Soc. Dairy Technol.*, 5 (3): 177. 1952.

RECENT DEVELOPMENTS IN AUTOMATIC CLEANING OF STORAGE TANKS[1]

V. E. MEYERS
Girton Manufacturing Company, Millville, Pennsylvania

Automatic cleaning means mechanical cleaning. When successful mechanical cleaning is being done it is possible, by instrumentation and control, to automatize the cleaning system to any desired degree. Different ideas seem to exist as to what sanitation means as it applies to dairy equipment. In this discussion we mean a tank that is clean as demanded by all sanitary standards. Several devices have been used to clean storage tanks by mechanical methods.

[1] Presented at Seventh Annual National Dairy Engineering Conference, Michigan State University, East Lansing, February 26-27, 1959.

We shall examine these devices and see how each one is supposed to operate.

EQUIPMENT

Spray balls. More spray balls have been installed in storage tanks than any other device. Some have been installed as a permanent part of tank equipment. Others have been designed to be placed in the tank when the cleaning operation is started. Both types of spray balls are expected to do the same cleaning job, so they will be grouped together. These spray balls have been placed on piping extending down from the top of the tanks, on horizontal piping welded to the tank liner, and on piping that rests on supports standing on the bottom of the tank. They have generally been self-draining. In fact, they need to be self-draining and self-cleaning if they are to be successful. Some spray balls are made in two sections which are separable and can be taken apart for cleaning. Other spray balls have a plastic ball installed inside, made to bounce around by the solution flow and keep the inside of the ball clean.

Recently, variations of the spray ball have been designed and installed in storage tanks. Generally, they are not spherical in shape, but are supposed to do the same job as the regular spray balls.

The spray balls and variations of them have given varying results. A pump to supply pressure to the cleaning solutions has been attached to the spray ball piping system. These spray balls have been used in all sizes and shapes of tanks. Generally speaking, a single spray ball has been installed for each 8 ft. of tank length. This has been a general rule, but is not true in all cases. They have been placed at different distances from the bottom and from the top of the tanks.

Hot sprays. The second device that has been used is the hot spray unit. This consists of a tank, hose, nozzle, and pump. A solution is prepared in the tank, and it is heated and sprayed on the tank surface. The hot solution is allowed to remain on the surface for a short time. A respray with the hot solution follows and then rinsing with fresh water as the last treatment. A man must enter the tank to apply the cleaning solution properly.

Injector sprays. The third cleaning device is the injector type of hot spray unit. This consists of a detergent reservoir tank, a Venturi element, and a mixing valve with hose and nozzle. The tank is filled with detergent and connected to a water supply. The water flows through the Venturi and draws detergent from the supply tank and mixes the solution. The nozzle is then controlled by the man who sprays the surface to be cleaned.

Rotary sprayers. The fourth device is the rotary type sprayer. It has a rotating head, driven by a motor, fitted with a number of spray nozzles. These nozzles are supposed to overlap and completely cover the inside surfaces of the tank. This sprayer requires a pump to circulate the cleaning solution through the spray nozzles.

Rotating nozzles. The fifth device consists of a rotating nozzle-equipped head. It is rotated by the jet action of one or more of the spray nozzles. This device has the spray patterns designed to overlap on the tank surface. The rotating mechanism has been made with sleeve type bearings and, more recently, with stainless steel ball bearings. This unit requires a fixed pressure system, to insure positive and constant speed of rotation. Usually, it has been installed in the top of storage tanks. Not many of these cleaning devices have been installed in tanks.

Rotating shafts. The sixth cleaning device consists of a rotating hollow shaft that extends down through the top of the storage tank. A fan-shaped, slotted jet is attached to the hollow shaft. Cleaning solutions, under pressure, flow through the hollow shaft and out through the slotted jet. Because the slotted jet throws a fan-shaped pattern of cleaning solution, an uninterrupted stream of solution is placed on the top surface of the tank liner. As the jet assembly rotates, complete coverage of the tank interior is accomplished with each revolution of the jet. Normally, this mechanism rotates five times per minute. This means that five times per minute the entire interior of the tank is covered with cleaning solution.

This rotating slotted jet has also been installed as an integral part of the agitator shaft. It is designed to allow normal use of the agitator without any adjustment. When the cleaning operation is started, solution is pumped through an inlet on top of the tank. The cleaning solution flows through the hollow shaft and out through the slotted jet. This fan-shaped pattern of solution covers the upper surfaces to be cleaned and flows down the sides and ends. An opening in the device allows solution to flow down the agitator shaft and thoroughly clean the shaft and upper blade or blades. The bottom agitator blade, or blades, and the bottom bearing, are cleaned by rotating in the pool of cleaning solution that collects on the bottom of the tank. This solution is drawn out through the outlet and discharged to the drain or used again.

These devices have been used for cleaning storage tanks, and different ideas exist as to their cleaning efficiency.

PROCEDURES

Probably the best way to clean a storage tank by mechanical means would be to proceed as follows:

1. Fill the tank with water, add the proper chemical to make a good cleaning solution, heat the solution to the proper temperature, and shake the tank until the soil is removed from the cleanable surfaces. The only problem is to

find a man strong enough to pick up a storage tank and shake it. By accepting this method as the perfect one, we can then consider these mechanical cleaning devices and see how they approach perfection. Certainly, it is safe to assume that good chemicals are available to do the cleaning job. Water and methods of heating are at hand. Pumps, tanks, and controls are all presently available. The different devices can all easily be installed in tanks. Now, all that remains is for the mechanical devices to completely cover the surfaces to be cleaned with cleaning solution.

The Spray Ball Method can do this, provided it is properly installed and if the proper pressure and volume of cleaning solution is supplied to the inlet. Also provided, and this is very important, that the storage tank has been designed to be cleaned by mechanical means. A spray ball, 4 in. in diameter, can have any number of holes drilled into its surface, through which the cleaning solution is discharged. To work at all, these balls must be some distance from the surface to be covered. Otherwise, all the solution would strike the surface in a small area. As the distance increases between the spray ball and the surface to be cleaned, the streams of solution get farther apart. This means that the solution has to bounce or slide across the area between these impact points. It is quite difficult to control this bounce or slide and, again, proper installation should be stressed. If the tank has a vertical agitator shaft, it is difficult to spray the back side of the shaft. It is hard to make water bounce, slide, or go around corners, into blind spots, bearings, or around any obstructions in the tank. The results from spray ball installations indicate that extreme care must be taken to insure that successful cleaning will be realized.

The hot spray system of cleaning tanks can be efficient, but it is still a manual operation. It requires the same attention to chemical solution, temperature, and time to insure success. This operation also requires that a man go inside the tank, which limits its usefulness as a mechanical method of cleaning storage tanks.

The injector system of hot spraying is essentially the same as the pump type system mentioned previously.

It should be mentioned that some air-agitated tanks have been fitted with pumps, to force cleaning solution through the air tubes, and that this was supposed to clean these tanks, but there is no information on the efficiency of this method. If complete coverage of the tank interior can be accomplished, this method could be successful.

The rotating head fitted with spray nozzles can be installed in tanks. This device will clean tanks, if it is supplied with the proper chemical solution, under the ideal pressure. This device has limitations. It can not spray around corners or behind shafts. It must be carefully engineered and installed. The nozzles must be adjusted to overlap and completely cover the soiled surface. The problem of being self-draining and sanitary in design must be met. Ordinary pipe threads on the nozzles must be removed and a sanitary connection made. This rotating nozzle-equipped head must be carefully installed in a tank to be efficient.

The self-rotating cleaning head has the same problems in cleaning tanks as do the other devices, and involves the design of the rotating mechanism. The bearings must be sanitary, self-cleaning, and self-draining. Cleaning solutions and the temperature of them affect the operation of this device. Because it is jet-operated, pressure affects its speed. This is another device that can clean tanks if all the engineering problems are solved and if it is properly installed in a tank that can be cleaned by mechanical means.

The slotted jet mechanism that is rotated by a separate motor will clean tanks with horizontal agitators or air agitation, if properly installed. The solution comes from the jet near the top of the tank. The jet puts solution on the top surface to be cleaned and into the sight-glass and vent openings. The solution flows down the sides of the tank to clean these surfaces. The device requires that a 4-in. sanitary ferrule be welded into the top of the tank. The mechanism attaches to this ferrule with a 4-in. sanitary nut. It is self-supporting and the motor is part of the unit. This makes a sanitary, easily inspected device, as the slotted jet comes apart for visual inspection.

The slotted jet device has given excellent cleaning results when installed on a vertical agitator shaft. The tank is cleaned because the slotted jet completely covers the inside of the tank, provided it has been designed to be cleaned by mechanical means.

Care must be exercised that this device is not rotated too fast. At 36 r.p.m. the device will clean a tank 16 ft. long. Longer tanks require slower-speed agitator motors. Two-speed motors have solved this problem.

Some tanks are not made to be cleaned mechanically. It is better to admit this fact and save trouble and misunderstanding when the proper results are not obtained.

From this evaluation of the available mechanical cleaning methods, it is apparent that a good practical method of cleaning storage tanks can be developed to a point where success is assured.

Along with the description of recent cleaning-device developments should go a description of cleaning methods as related to chemicals, time, temperature, rinsing, sanitizing, and volume of solutions required. Much work has been done along these lines and some practical answers to the questions have been developed.

Prerinsing. We have found that a good prerinse with water at 90 to 100° F. is the best way to remove the loose soil in storage tanks. Applying three-tenths of a gallon to each square foot

of tank surface will result in good prerinsing. This figure is correct with a slotted, fan-shaped jet. A good practical way of deciding on the amount of prerinse water to use is to observe the water as it leaves the tank outlet. When it is clear, the tank is adequately rinsed.

Chemicals. We recommend that the advice of chemical manufacturers be followed. These people know their products and what they will do. They have the materials to properly clean these tanks by mechanical means, and they should be consulted in their use. The solution must be strong enough to remove the soil in the tank, but excessive chemicals result only in waste. We feel that the solution temperature should be kept as low as possible. However, the chemical in question may require a minimum temperature, so it is best to follow recommendations. Remember that heat causes the stainless steel to expand, cold causes it to contract. These forces should be held in check. Results have proven that cleaning can be accomplished by a maximum solution temperature of 135° F. The slotted jet delivering 50 g.p.m. to the interior surfaces of the tank will clean a 2,000-gal. storage tank in 10 min.

A cooling fresh-water rinse should be applied after the cleaning solution treatment. This removes all traces of the solution and cools the tank surfaces. Good rinsing is essential to good cleaning.

DISCUSSION

Mechanical cleaning to be successful depends on these factors: complete coverage of the surfaces to be cleaned with a chemical solution of the proper concentration, heated to and maintained at the proper temperature. The solution must be kept in contact with the tank surfaces for the prescribed length of time. Coverage, time, temperature, and solution concentration are the things necessary to insure clean tanks.

An important side-effect to consider when cleaning storage tanks is the cooling system on the individual tank. Hot solutions contacting the refrigerated zone can cause much grief, if the proper precautions are not followed. It is essential that a refrigeration engineer be consulted before proceeding with mechanical cleaning.

From developments up to this time, it is apparent that we can clean storage tanks by mechanical means. The system can be automatic to any desired degree. Installing an automatic circulation cleaning system insures that time, temperature, and solution concentration are maintained. Installing flow-diversion valves in the lines to and from the tanks to be cleaned, and applying controls and indicators to them, will make a system push-button in operation.

In other words, pushing button No. 1 would clean Tank No. 1 automatically. All precautions would be observed by the control system. If the tank was not empty, a control would not allow the cleaning operation to begin. Anything desired can be built into the automatic system.

These recent developments have indicated that a practical method of cleaning tanks mechanically has been developed to the point where success is assured.

These developments have proven that mechanical cleaning is being accepted by the industry. It is important to build storage tanks with mechanical cleaning as the main objective. This requires some thorough planning by the designers and means that we must consider new ideas about the construction of storage tanks. It is very difficult to apply a good mechanical cleaning device to a tank built years ago for hand-cleaning.

The outlet of the storage tank can generally be cleaned mechanically. The manhole, door, and gasket on present tanks must be washed by hand. This is true when mechanical cleaners are used. The sight-glass opening on most tanks has to be removed and hand-washed; the same is true of the inlet and air vent openings. The test valve, the measuring device on the tank, and the agitator bearing must be cleaned by hand. Therefore, it is apparent that consideration must be given to the redesigning of tanks. It is possible to build a storage tank now that can be cleaned mechanically. This could be done by moving the manhole to the top of the tank. The manhole would be used only for inspection and could be much lighter and would be out of the product zone. The air vent and the product inlet could be in this door or, in many cases, the product could enter the tank through the outlet valve. This method would remove the product inlet and its cleaning problem. A practical method of measuring the volume of liquid in the tank is available and it is known as the bubble system; it requires only a small sanitary C.I.P. fitting on the bottom of the tank. The indicating thermometer fitting is no problem. The test cock may have to be cleaned by hand, if it is necessary to have one on the tank. If a sight-glass is necessary, it can be of the new flush-mounted cleaning-in-place type that is easily cleaned. The vertical agitator has the cleaning device mounted as an integral part and is self-cleaning and its support bearing is also self-cleaning.

When tanks are built along these lines, mechanical cleaning will be easy and positive results will be obtained. These cleaning devices are developed and are practical. Storage tanks must be built to take advantage of these labor-saving methods.

OUR ASSOCIATION

CALL FOR PAPERS FOR THE 1960 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

N. L. JACOBSON, Chairman, Program Committee
Department of Animal Husbandry, Iowa State College, Ames

The 55th Annual Meeting of the American Dairy Science Association will be held June 19-22 at Utah State University, Logan. Members who wish to present papers must submit titles and abstracts not later than March 1. This is a change from last year. The new deadline must be met to permit publication of titles in the May issue and abstracts in the June issue of the JOURNAL.

Abstracts must contain no more than 200 words, arrived at by counting each typed line as 12 words. Abstracts longer than 200 words will be returned to the author(s) by the Committee. An original (on bond paper) and three copies of each abstract should be typed double-spaced. The original and one copy should be mailed to the Chairman, another copy to the Vice-Chairman, and the fourth to the Secretary of the Section before which the paper is to be presented.

In the preparation of abstracts, the style and abbreviations of the JOURNAL OF DAIRY SCIENCE must be used. Please refer to abstracts in the May, 1959, issue of the JOURNAL for guidance. The title of the abstract should indicate clearly the nature of the research. The abstract should include, insofar as possible, the design and major results of the study. Only research which has been completed should be reported. Brief, essential statistics will make the data more meaningful.

All members of the Association are encouraged to present papers. Participation by members in the industry and by senior members of the Association is particularly welcome. The competitive presentation of papers by graduate students, initiated in the Production Section in 1959, will be extended both to the Manufacturing and to the Production Sections in 1960.

Abstracts of research accepted for publication by a scientific journal before the annual meeting are not acceptable. The Program Committee favors the general policy that an individual present only one paper and that his name appear as author on no more than two. The Program Committee, with the Association members, desires to stimulate vigorous, sound research and realizes that the above restrictions may penalize some members engaged in full-time research. Therefore, the Committee has liberalized this policy so that a member can exceed these limits, but only if he or his department rates the abstracts in order of preference for oral presentation. If the total number of papers submitted by the membership is too great to include in the program, the Committtee will assign some papers to be read by title only.

The Program Committee encourages continued improvement in quality of papers and in oral presentations. Careful design of experiments and proper interpretation of results are necessary prerequisites. Strict compliance with the instructions for preparation of abstracts will simplify the task of the Program Committee and will improve the program. Careful editing of abstracts before submission is essential.

Preparation of mimeographed copies of pertinent data is desirable. At least 250 copies should be available for distribution. Material for projection on screens should be used only if mimeographing is impractical.

Names and addresses of officers of sections to whom titles and abstracts should be sent are:

EXTENSION SECTION

Chairman: C. W. Nibler, Department of Dairy Husbandry, University of Nebraska, Lincoln.

Vice-Chairman: M. E. Senger, Department of Animal Industry, North Carolina State College, Raleigh.

Secretary: W. R. Van Sant, Department of Dairy Science, University of Arizona, Tucson.

MANUFACTURING SECTION

Chairman: Stuart Patton, Department of Dairy Science, Pennsylvania State University, University Park.

Vice-Chairman: Burdet Heinemann, Producers Creamery Co., Springfield, Missouri.

Secretary: M. L. Speck, Department of Animal Industry, North Carolina State College, Raleigh

PRODUCTION SECTION

Chairman: G. W. Trimberger, Department of Animal Husbandry, Cornell University, Ithaca, New York.

Vice-Chairman: S. D. Musgrave, Department of Dairying, Oklahoma State University, Stillwater.

Secretary: J. C. Thompson, Ralston Purina Co., St. Louis, Missouri.

NOMINATIONS FOR VICE-PRESIDENT AND DIRECTORS OF A.D.S.A.

D. V. JOSEPHSON, Chairman, Nominating Committee
Department of Dairy Science, Pennsylvania State University, University Park

The By-laws of our Association relating to the nomination of officers were published on page 4 of the October, 1958, issue of the JOURNAL. The By-laws specify that the membership be invited to suggest names of competent individuals from among the members of A.D.S.A. for the consideration of the Nominating Committee.

This Committee feels that it can not do an effective job of preparing a slate of officers without the assistance and full cooperation of the membership. Our Association needs strong, aggressive leadership and much care must be taken in selecting this leadership. Therefore, all members are urged to suggest the names of prominent, competent members who are capable of filling and willing to fill these important offices of our Association.

This year we must elect our Vice-President from the Dairy Manufacturing area. In making your suggestions it should be borne in mind that, if elected Vice-President, your nominee will succeed to the Presidency the following year. For this reason, it will be helpful if your suggested candidate be a man with known administrative capacity and experience. Other factors that might be considered are: service as a Director of A.D.S.A., Section Officer experience, Section or Association Committee leadership, and known devotion to A.D.S.A. objectives.

The two new Directors of the Association for the coming year will be selected as follows: One from the Manufacturing and one from the Production group. Our present group of six Directors includes two from the Extension Section whose terms continue through next year. In accordance with established policy, no one from this latter area will be considered for the office of Director this year.

In recommending candidates for Director, the nominator should consider people who have demonstrated a devoted interest in A.D.S.A. affairs. Members who have held section offices, committee chairmanships, or other Association assignments should be given preference. Known administrative capacity also should be considered.

All suggestions for officers should reach the Chairman of the Nominating Committee by January 1, 1960. The Chairman will assemble the lists and then request a biographical sketch from one of the nominators of each candidate. The Committee will then consider these lists and the supporting information in arriving at the final slate of two candidates for each office, whose names will appear on the election ballot.

The Committee urges the membership to consider this matter seriously. As a Committee of only five men, we are not in a position to recognize all of the qualified people who should be considered for these offices. The future strength and progress of A.D.S.A. will depend upon the leadership we elect. For this reason, we need the help of all members in keeping the offices of the Association filled with the most qualified and devoted people from among our membership.

REPORT OF REPRESENTATIVE TO THE UNITED STATES LIVESTOCK SANITARY ASSOCIATION

C. G. BRADT
Department of Animal Husbandry, Cornell University, Ithaca, New York

The sixty-second annual meeting of the United States Livestock Sanitary Association was held at Miami Beach, Florida, November 4–7, 1958. Florida was selected as the meeting place this year as a mark of recognition of the growing livestock industry of the South. While the attendance was not as large as at previous meetings, in the Midwest, about 150 persons from all over the country were present. It was a splendid conference, with many excellent papers and committee reports presented.

It was my privilege to be the appointed representative of the American Dairy Science Association at this meeting. While there, the writer served as a member of the Committee on Infectious Diseases of Cattle and on the Committee on Brucellosis at the request of W. D. Knox, a regular committee member who was unable to be present. Both committees presented carefully prepared reports to the general assembly after spending much evening time in discussion prior to their final formulation.

The Infectious Diseases of Cattle Committee emphasized the need for special consideration and study on the problems of genital diseases (with particular attention to nonspecific abortions), anthrax, mastitis, salmonellosis and virus diarrhea or mucosal disease. Special situation reviews included in this committee report covered listeriosis, mucosal disease complex, nocardial mastitis (a newly identified udder disease in California), and vibriosis.

The Brucellosis Committee presented a number of amendments to the present regulations now being observed and followed in the National Brucellosis Program. A plan for nationwide swine brucellosis eradication was prepared. It was believed that with cattle brucellosis now being brought under control and near-eliminated in many states, brucellosis in swine as a source of infection for cattle and man should be vigorously attacked. It was stated that surveys in the United States show about 5% of the hog population infected or suspect, with 2% infected.

ANAPLASMOSIS, A PROBLEM IN MANY STATES

Two papers and a committee report were presented upon the topic of Anaplasmosis. The complement-fixation test was discussed, and treatments with chlortetracycline mixed in cottonseed meal and given orally according to body weight for 60 days have given good results. All cattle treated were negative by complement-fixation test 60 days after treatment and, under field tests on the farm, 83% were negative according to Oklahoma experiments. To save valuable cattle, such treatments, while expensive, are practical.

Anaplasmosis was reported as being diagnosed in sheep in Wyoming, but was believed not to be transmissible to cattle. In California, blood of infected deer was inoculated into 18 calves. Twelve developed the disease within 14 to 28 days later, but none died. Anaplasmosis research is going on in 12 states and at Beltsville. A test-and-slaughter program in Hawaii is giving favorable eradication results under the conditions prevailing there.

LEPTOSPIROSIS COMMITTEE GIVES ADVICE

Leptospirosis in wildlife animals was discussed by H. E. Goldstein of the Ohio Animal Disease Laboratory, and current diagnostic problems were outlined by E. V. Morse of Ames. Surveys in Ohio indicate that of 932 wildlife blood samples tested by the lysis test, deer samples were 20% positive, foxes 25%, raccoons 23%, and skunks 54% positive, 1 to 100 or higher. The point was made that more research is needed relating to the part infected wildlife plays in the spread of this disease in domestic animals.

In discussing leptospirosis tests, Morse stated that leptospirosis positive titers indicate past or present infection. Some cross-reactions may be observed, but they do not become a significant problem. Vaccines may produce reactions for short periods, but usually disappear. Leptospirosis should be considered as a herd problem when a blood test interpretation is being considered.

The Committee on Leptospirosis in its report lists the following points:

1. The test is only an aid in diagnosis. Clinical symptoms should be noted, also.
2. Vaccinate all negative animals and all herd additions.
3. Some vaccinates may abort due to being in the incubation stage at time of vaccination.
4. Vaccinate show-ring cattle prior to the show season.
5. Vaccination applied to infected cattle is of no value.
6. A uniform test is needed.
7. No state program for elimination can be advised until a more specific test has been developed.

SOME OTHER CATTLE DISEASES REVIEWED

Bovine encephalomyelitis, a virus disease of beef and dairy cattle, has been diagnosed in 12 California counties, reported J. B. Enright of Davis. Both sexes may be affected. The disease seldom spreads outside the herd. However, the mode of transmission at present is unknown. Some abortions in beef herds have been noted and an acute form of mastitis in dairy herds is sometimes a symptom. Animals move with a stiff, unsteady gait; they stumble, and hock joints may show swellings. Animals go off-feed, lose weight, and are depressed. Poor condition may persist for a long period and some cattle may die in convulsions, but others recover. Certain antibiotics have been found useful as treatments.

Pustular vulvovaginitis in cattle was reported upon by J. H. Gillespie of Cornell. He said that 15% of the dairy herds in New York, according to his data, have this disease. In one herd of 90 cows in northern New York, 75% were infected. Research indicates that this disease and rhinotracheitis are caused by the same infective agent.

Johne's disease came up for discussion in a paper delivered by A. B. Larson of Auburn, Alabama. Of the johnin tests, he said the intradermal test appears to be the best, according to experiments. It has been found that some adult reactors do become negative later, but the number of recoveries seems to be fewer than with young animals.

The Committee on Vesicular Disease (Foot-and-Mouth, vesicular exanthema, and vesicular stomatitis) reported that 95% of all garbage fed in the United States is now being cooked. All but two states have cooking laws in force.

States were advised to increase funds for garbage inspection, as United States government support is being curtailed for this service.

All cases of vesicular stomatitis should be thoroughly checked, to be certain that none are Foot-and-Mouth disease. Research at Plum Island continues, as does the project on vesicular stomatitis in the coastal region of Georgia, where 79% of the feral swine show antibodies on tests.

PARASITE CONTROL REPORTED UPON

R. C. Bushland from the Kerrville, Texas, Research Station reported upon the work with systemic treatments. Many products by Bayer, Dow Chemical, and American Cyanamid are under investigation. For cattle grubs, neither Bayer nor Dow products give 100% control. They are expensive, too. Treatment for a big bull may be as much as $3. Spray materials are cheaper, it was pointed out. Products to control flies, ticks, and screw-worms, used orally and subcutaneously, also are under investigation at Kerrville.

Some cattle ticks are found in Florida, but are being well controlled by dipping and quarantines. Texas and California also have them along the Mexican border.

The Screw-worm Eradication Program centering at Sebring, Florida, was reported upon by R. S. Sharman of the United States Department of Agriculture. An excellent set of slides was shown of the Sebring operations, in the production and distribution of sterilized male screw-worm flies over Florida, South Georgia, and Southern Alabama. The results to date have been highly successful in the eradication of this animal pest, which has been known in one case to kill a grown steer in ten days.

BOVINE TUBERCULOSIS NOT YET ERADICATED

The Tuberculosis (TB) Eradication Committee made the following recommendations:

1. An increase in cattle testing is needed.
2. The TB reactor tracing service at sales stables and stockyards should be extended.
3. Veterinary colleges should emphasize in their curriculums the importance of TB eradication programs and procedures.
4. More education of cattle owners to combat growing complacency is needed.
5. Maintain rigid quarantines of infected herds.
6. Eradicate TB in other species, especially poultry.
7. Eliminate the no-visible-lesion (NVL) problem through continuing research.

It was stated that 243 counties in the United States are overdue for TB tests.

NEW LEGISLATION, LAWS AND REGULATIONS

The Legislative Committee recommended increased funds for the United States Department of Agriculture for the meat inspection service and for checking the value of new vaccines and biologics. Better control of wild animal imports from abroad was advised.

Uniform state rules and regulations to facilitate interstate livestock movement, and to keep reports of these regulations up to date when amended, were suggested by the Committee on Laws and Regulations.

Officers elected for 1959 are: President, F. G. Buzzell, Augusta, Maine; First Vice-President, James R. Hay, Columbus, Ohio; Second Vice-President, A. P. Schneider, Boise, Idaho; Third Vice-President, W. L. Bendix, Richmond, Virginia; Secretary-Treasurer, R. A. Hendershott, Trenton, New Jersey.

Each year, a complete record of all the papers and committee reports presented at the annual meeting of the Association are published in the *Proceedings*, a neatly printed book of about 350 pages. All members are mailed copies without additional cost.

Membership in the Association is open to any person engaged in livestock sanitary work for federal, provincial, state, territory, county, or municipal governments and to any other person interested in livestock sanitation or milk and meat hygiene.

SOME CURRENT ASPECTS OF ANIMAL DISEASE CONTROL

Report of a Work Party of the Public Health Committee of the American Dairy Science Association for 1959

L. A. BLACK
Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio
AND
W. D. POUNDEN
Ohio Agricultural Experiment Station, Wooster, Ohio

Preliminary reports for 1958 by the National Office of Vital Statistics reveal there were 12% fewer human cases of brucellosis than in 1957. At least part of this accomplishment must be attributed to the national brucellosis eradication program in cattle (5). Recent reports indicate that tuberculosis in cattle is undergoing a slight rise in incidence in some areas of the central states and, because of its relationship to dairy herds, no doubt greater emphasis will be expended on its control. This slight rise in tuberculosis also indicates that more frequent testing may be desired from the viewpoint of public health in respect to sanitary milk control, and that such testing might be done at a 3-yr. interval. Leptospirosis apparently is quite prevalent in many areas and may require considerable effort to prevent its being a recurring problem. Although the organisms are killed by pasteurization, danger may exist to personnel working with infected herds.

Q FEVER

Q fever, a rickettsial disease known to occur throughout the world, has been endemic in cattle, goats, and sheep in certain areas of the western and southwestern states. Preliminary studies and reports by the Rocky Mountain Laboratory of the National Institute of Allergy and Infectious Diseases at Hamilton, Montana, (6), in cooperation with state health department and agricultural agencies, indicate the occurrence of Q fever infections in areas hitherto presumed free from the disease. For example, "We now know that bovine Q fever occurs in every section of the United States. It is especially prevalent in the East, where from 25% to 70% of herds within several states are infected and, undoubtedly, excreting rickettsiae into the environment in milk. We have yet to find a state free of infection! The full implications of all this are not adequately understood, but they are alarming, since the infections which are spreading are the only sources for infection in man. Thus, there is today a need for extensive testing of milk supplies for evidence of bovine infections, to determine the need for local considerations of the disease as a potential hazard to people. Little testing is being done, on milks or human serums, and that being done is experimental studies being sparked from here."

Apparently then, bovine infections with the rickettsiae *Coxiella burnetii* occur in all parts of the United States and are a source of undetermined amounts of human infection and illness. The organisms are excreted in milk and in very large quantities in placental tissues and fluids. The asymptomatic animal infections which are spreading may constitute an increasing public health problem. Human cases of Q fever are associated with casual or occupational exposure to infected livestock and contaminated premises, from residence near infected premises, or from household use of raw milk. Although pasteurization of milk at 145° F. for 30 min. or at 161° F. for 15 sec. destroys the agent (2), an additional 5° higher temperature has been recommended for processing products containing high fat or added sugar. In areas where Q fever has not heretofore been recognized, some dairy products may not receive sufficient heat treatment to destroy such organisms. Where the additional heat treatment recommended by health officials (2) is utilized, this also results in added destruction of the phosphatase enzyme, and thus phosphatase test levels of properly pasteurized products should be well below currently acceptable standards.

STAPHYLOCOCCAL FOOD POISONING

In recent years, outbreaks of disease associated with milk and dairy products have occurred relatively infrequently, ordinarily less than ten per year. Nevertheless, since many people may be involved, these assume great importance. Outbreaks of food poisoning associated with staphylococci in dairy products have been reported in recent years and in 1958 several outbreaks were reported due to natural cheese. In one outbreak, a total of 66 cases was investigated epidemiologically, and bacteriophage types were determined on the coagulase-positive hemolytic staphylococci isolated from cheese. Examination of 24 samples of such cheese showed that all had concentrations of hemolytic staphylococci greater than one million organisms. In another outbreak, hemolytic coagulase-positive staphylococci were isolated from natural Cheddar cheese served to a group of 200 persons who became ill (4).

Although there has been much discussion of staphylococci which are resistant to common antibiotics, the instances when these have been

picked up in association with udders have been surprisingly few (10, 11). However, as long as resistant strains exist there is always the chance that they will be involved in mastitis problems. The finding of preformed staphylococcus enterotoxin in spray-dried milk illustrates the fact that staphylococcus enterotoxin is heat-stable and may be encountered in milk products containing but a few staphylococci if, prior to their destruction by processing, conditions permitted their growth and development of toxin (12). Since such organisms may grow rapidly in pasteurized milk and milk products at 80–120° F., prolonged holding at these temperatures after pasteurization should be avoided.

SALMONELLA

In 1958, an outbreak of *Salmonella dublin* infection was traced to certified raw milk, with 11 laboratory-proved and 19 suspect cases. Three isolations of Salmonellae organisms were made from cows in the herd of about 400 animals (3). In this connection, it may be of interest to note that a working party of the Public Health Laboratory Services in England has found Salmonella present in a wide variety of raw, processed, and complete feeding meals, especially imported bone, bone products, and fish meal. Although viable counts were low (less than 100 per 100 g.), 88 serotypes were isolated, and further work was planned as to their epidemiological importance (9).

YEASTS, ACTINOMYCETES, AND VIRUSES

Udder infections due to yeasts and yeastlike fungi, such as Cryptococcus (8), and actinomycetes such as Nocardia (7), can present some potential problems of human involvement. The aerobic actinomycetes which are pathogenic for humans have been isolated from mastitis in cattle in various sections of this country. Their biology is not very well understood; virulence differs, so that infections may vary from almost inapparent illness to severe mastitis, and some strains of the organism show a marked resistance to heat as isolated from the milk of infected cows. The organisms are resistant to most antibiotics and their use is more likely to aggravate than to help the problem. The finding of this organism in the udders of dairy cattle should be of considerable public health importance and requires further study.

The isolation of members of the lymphogranuloma venerum group of viruses from humans and almost all kinds of livestock, including cattle, indicates the tremendously broad areas in which these infections are involved. Perhaps of some significance is the finding that some of these viruses can exist in udders (1) and can be shed from them.

REFERENCES

(1) Bannister, G. L., Boulanger, P., and Rice, C. E. Mastitis Produced Experimentally in a Cow with an Agent of the Psittacosis-Lymphogranuloma Group of Viruses. *Canadian J. Comp. Med. Vet. Sci.*, **23**: 47. 1959.

(2) Enright, J. B., Sadler, W. W., and Thomas, R. C. Thermal Inactivation of *Coxiella burnetii* and Its Relation to Pasteurization of Milk. *Public Health Rept.*, **72**: 947. 1957. (Public Health Monograph No. 47.)

(3) Epidemiological Reports. Salmonellosis. *Morbidity and Mortality Weekly Rept.*, **7**: 2. December 27, 1958.

(4) Epidemiological Reports. Staphylococcal Food Poisoning from Cheese. *Morbidity and Mortality Weekly Rept.*, **7**: 2. December 19, 1958.

(5) Held, J. R., Bauer, H., and West, R. L. Effect of Eradicating Brucellosis in Cattle on Incidence of Human Cases. *Public Health Rept.*, **73**: 1096. 1958.

(6) Luoto, L. Personal communication. February 10, 1959.

(7) Pier, A. C., Gray, D. M., and Fossatti, M. J. *Nocardia asteroides*—A Newly Recognized Pathogen of the Mastitis Complex. *Am. J. Vet. Research*, **19**: 319. 1958.

(8) Pounden, W. D., Amberson, J. M., and Jaeger, R. F. A Severe Mastitis Problem Associated with *Cryptococcus neoformans* in a Large Dairy Herd. *Am. J. Vet. Research*, **13**: 121. 1952.

(9) Salmonella Organisms in Animal Feeding Stuffs and Fertilizers. Report of a Working Party of the Public Health Laboratory Service, Monthly Bull., Ministry of Health, and the Public Health Laboratory Service, *Med. Research Council*, **18**: 26. February, 1959.

(10) Seto, J. T., and Wilson, J. B. Bacteriophage Typing of Micrococci of Bovine Origin. *Am. J. Vet. Research*, **19**: 241. 1958.

(11) Smith, H. W. The Multiplication of *Staphylococcus aureus* in Cows' Milk. *Monthly Bull.*, Ministry Health, **16**: 39. 1957.

(12) Takahashi, I., and Johns, C. K. *Staphylococcus aureus* in Cheddar Cheese. *J. Dairy Sci.*, **42**: 1032. 1959.

ABSTRACTS OF PAPERS

PRESENTED AT THE WESTERN DIVISION MEETING

UNIVERSITY OF ARIZONA, TUCSON
JULY 13 TO 15, 1959

Mastitis Studies in a Dairy Herd. GAYLE GILDA AND E. GRANT MOODY, Arizona State University, Tempe.

A 7-yr. study of 254 separate incidents of mastitis in 87 cows in the Arizona State University herd was made. An attack of mastitis resulted in the immediate loss of more than 10 lb. of milk. The decrease in production occurred 24 hr. before the appearance of clinical symptoms. The incidence of mastitis in a herd was drastically reduced by proper management and the culling of cows with chronic mastitis. Bacteriological analysis of secretions from 15 cows indicated that *S. aureus*, the most commonly occurring organism, showed a measure of in vitro sensitivity to all antibiotics tried except polymixin.

Dried Molasses Beet Pulp vs. Chopped Alfalfa Hay as a Preservative for Oat and Vetch Silage. D. D. BULLIS, R. H. KLIEWER, AND I. R. JONES, Oregon State College, Corvallis.

Twenty-four Holstein and Jersey cows, paired according to breed, age, body weight, production, stage of lactation, and gestation were fed oat and vetch silage to appetite, preserved with 200 lb. of dried molasses beet pulp or chopped alfalfa hay. No significant difference ($P < 0.05$) in FCM, body weight change, or FCM produced per pound of dry matter consumed was observed. However, there was a significant ($P > 0.01$) difference in silage dry matter consumed per day per cow and a significant difference ($P > 0.02$) in silage dry matter consumption per pound of body weight in favor of the molasses beet pulp preserved silage.

There was less plant juice loss, less top spoilage and, therefore, less total dry matter loss with the dried molasses beet pulp preserved silage than with the chopped alfalfa hay preserved silage. The 7,500 lb. greater juice loss with the chopped alfalfa hay silage may be conservatively estimated to contain 8% dry matter, or an apparent saving of 600 lb. of dry matter in favor of the dried molasses beet pulp preserved silage.

Ensiled forage preserved with chopped alfalfa hay was found to be higher in protein, fiber, fat, and ash than the same forage preserved with molasses dried beet pulp. However, the latter silage had the highest N.F.E. and TDN values.

Comparative Feeding Value of Oat and Pea Silage Preserved with Dried Molasses Beet Pulp vs. Ryegrass Screenings. D. D. BULLIS, R. H. KLIEWER, AND I. R. JONES, Oregon State College, Corvallis.

The feeding value of high-moisture oat and pea forage preserved with ryegrass screenings or dried molasses beet pulp at the rate of 200 lb. per ton was compared, using 22 Holstein and Jersey cows paired into two groups and fed to appetite. A 4-wk. Latin-square crossover experimental design was used with a 4-wk. preliminary period and a 1-wk. change-over period between treatments.

Cows in each group consumed significantly more ($P < 0.01$) molasses beet pulp preserved silage (13.20 lb. more per day per cow), but failed to show a significant increase ($P > 0.05$) in energy-corrected-milk (ECM) compared to the rygrass screenings preserved silage. However, a significant increase ($P < 0.05$) in body weight for those animals fed the molasses beet pulp preserved silage was shown, reflecting the increase in consumption.

Effect of Feeding Low Levels of Chlortetracycline upon the Health and Milk Production of Dairy Cows. R. H. ROSS, D. L. FOURT, J. W. BAILEY, AND D. L. THACKER, University of Idaho, Moscow.

Twelve Holstein cows paired into two groups were used to determine the value of feeding 0.1 mg. chlortetracycline per pound body weight upon milk production, mastitis, bloat, and footrot. A reversal trial consisting of four 4-wk. periods was used. An additional 48 cows were used to study the effect of chlortetracycline upon mastitis, bloat, and footrot.

Feeding chlortetracycline had no effect upon milk production, mastitis, bloat, or footrot. Milk production was 37.5 lb. and 37.7 lb. 4% FCM for the control and experimental groups, respectively. There was one case of footrot in the control group. There were five cases of bloat in each group. Chronic cases of mastitis continued in both groups and no new cases developed in either group.

Alfalfa "Hayfers" vs. Baled Alfalfa Hay for Milk and Butterfat Production. R. H. ROSS, D. L. FOURT, AND F. C. GEPHART, University of Idaho, Moscow.

Twelve Jersey cows paired into two groups were used to compare baled alfalfa hay and "hayfers." The alfalfa hayfers were made by compressing chopped alfalfa hay into wafers about 4 in. in diameter and 1 in. thick. One cutting of hay from the same field was used

for both lots of hay. No supplemental feeds were used except iodized salt and bone meal fed free-choice. Per cent butterfat in the milk was determined on three-day composite tests taken continuously. A reversal trial was used, consisting of two, 5-wk. periods.

Mean daily milk production was 19.9 lb. 4% FCM for both groups. There was a significant reduction in butterfat test ($P < .05$). Baled hay averaged 5.74% and hayfers 5.48% butterfat, a reduction of 0.26%. The cows while receiving hayfers ate 27.7 lb. and wasted 1.6 lb. hay per cow per day and gained 0.74 lb. body weight per cow per day. While receiving baled hay, they ate 22.0 lb. hay and wasted 7.0 lb. per cow per day and lost 1.04 lb. body weight per cow per day. These differences were significant ($P < .05$).

Milk and Butterfat Production from Mash and Pelleted Grain Rations. LELAND TIGGES AND G. M. WARD, Colorado State University, Fort Collins.

The same ingredients were prepared as a coarsely ground grain mix and as 3/8-in. pellets and fed to 27 purebred Holstein-Friesian cows in the Colorado State University herd. The grain was fed to each group at the rate of approximately 1 lb. per 4 lb. of 4% FCM. Alfalfa hay and corn silage was group-fed in outdoor mangers. All cows were milked in a six-cow elevated milking parlor.

The study contained four trials consisting of a ten-day preliminary period followed by a 20-day experimental period. The order of trials was (I) pellets, (II) mash, (III) pellets, and (IV) mash. The average daily milk production and butterfat percentage for each period was, respectively: (I) 43.7, 3.39; (II) 42.4, 3.59; (III) 40.8, 3.31, and (IV) 38.6, 3.34. Differences between the two rations in milk production, fat test, and 4% FCM were not statistically significant, although the lower fat test obtained with the pelleted grain approached significance at the 5% level. All cows were able to finish eating their allotted grain in either form during an average of 7 min. which they spent in the milking parlor.

A Summary of Three Years' Work with Holstein Steers. R. O. RUPP, G. M. WARD, MELVIN H. HAZALEUS, AND H. B. LINDHOLM, Colorado State University, Fort Collins.

During the past 3 yr., over 100 Holstein steers were raised on various feeding systems and marketed at different ages and weights. From this study, the following conclusions can be drawn:

1. Veal production from dairy calves generally is not profitable in Colorado.
2. Cattle raised to an age and weight of feeders (500-700 lb.) were generally more profitable than the same animals fed to 1,000 lb.
3. Steers fed on straight roughage or roughage plus a limited amount of grain will make rapid gains and yield carcasses generally grading U.S. Standard.
4. All roughage rations require a 50- to 60-day longer feeding period to produce the same weights and carcass grades as steers fed grain at the rate of about 10 lb. per day.
5. All roughage feeding resulted in the lowest costs per pound of gain.
6. Dried beet pulp can replace 90% of the roughage of a normal hay and grain ration for a period of 130 days. This system gave the fastest gains and the highest-grading carcasses.
7. Stilbestrol or hormone implants increased gains and feed efficiency comparable to values reported for beef breeds.

Animal Fat as a Replacement for Barley in Dairy Cattle Rations. J. F. STEIN, M. A. WADE, D. W. CASSARD, AND L. WALKER, University of Nevada, Reno.

Sixteen lactating Holstein cows were divided into four comparable groups. Two concentrate mixtures were fed. The experimental mixture contained approximately 10% stabilized animal fat and was formulated on the basis of substituting 1 lb. fat for 2.5 lb. of barley in the control mixture.

In a change-over design, each group remained on each of the following treatments for a period of 5 wk. with a seven-day transition period between treatments: (I) Control fed at the rate of 1:6 FCM; (II) fat-concentrate at 1:6 FCM; (III) fat-concentrate at 1:8 FCM; (IV) fat-concentrate pelleted and fed at 1:8 FCM. Adjustments were made in the rate of feeding at the beginning and midway in each treatment period. Each group received good-quality alfalfa hay ad libitum under dry-lot feeding conditions. The mean daily pounds fat intake from concentrates (D.M. basis) were 0.14, 0.60, 0.45, and 0.50, respectively.

Mean daily pounds FCM produced on the various treatments were (I) 35.4, (II) 38.5, (III) 36.7, and (IV) 37.5. Feeding the fat-concentrate (II) resulted in a significant increase ($P < 0.01$) in FCM production over (I). No significant differences in body weight of animals were noted between treatments.

Feeding the fat-concentrate (II), (III), (IV) resulted in a significant increase in plasma lipid value ($P < 0.01$). No significant differences in plasma glucose and ketone bodies were observed. These values were in the normal range as reported in the literature.

Pineapple Plant as a Feed for Dairy Cows. K. K. OTAGAKI AND K. MORITA, University of Hawaii, Honolulu.

There is a lack of forage for milking cows supplying the Honolulu milk market because

of lack of agricultural areas for economical forage production. Pineapple plants are readily available in tremendous quantities, but only meager information is available on their nutritive value. Twelve Holstein-Friesian milking cows were allotted to three treatments in a double change-over design with 5-wk. periods. All cows received the same basal ration according to size and production and were supplemented with chopped Napier grass, ensiled or fresh pineapple plant to the limit of appetite. There were no significant differences between treatments in actual or fat-corrected milk production. Highly significant differences were noted in butterfat tests between cows within groups and between treatments. Some of these differences may be attributed to significantly lower dry matter intakes on fresh and ensiled pineapple plant regimes. There were no significant differences in milk flavors due to treatments. There were no indications of any adverse physiological effect from feeding pineapple plants as a source of roughage to lactating cows. Digestibility studies of fresh pineapple plant showed a high degree of digestibility of the crude fiber fraction. The DCP and TDN values for fresh pineapple plant were 6.8 and 74.8%, respectively, on the oven-dry basis.

Relationship of Bacteria and Progesterone to the Androgenic Activity of Cow Feces. C. E. LUEKER, University of California, Davis.

An investigation was designed to study the paradoxical fact that the feces of the female bovine contain a greater androgenic activity than bull feces. In the course of this experiment, the possibility of bacterial action being responsible for the androgenic property of incubated cow feces was studied. It was shown by the chick comb growth assay method that no androgenic response was obtained when cow feces were sterilized before they were incubated. However, when cow feces were sterilized, inoculated with fresh cow feces and then incubated, an androgenic response was obtained. The androgenic property found in incubated feces from female cattle appears to be the result of the conversion of a nonandrogenic material to an androgenic substance. Addition of progesterone to cow feces before incubation increased the androgenic activity. Thus, the conversion of progesterone to an androgenic substance by bacterial action provides a plausible explanation for the presence of androgenic activity found in cow feces.

Feeding Value and in Vitro Performance of Live Yeast Cultures. HERBERT JORDAN AND G. M. WARD, Colorado State University, Fort Collins.

A live culture yeast supplement added at the rate of 40 lb. per ton of grain was fed to eight Holstein cows and compared to a control ration of the same grain mix without the supplement. Data were recorded for five periods of 2 wk. each: (I) control, (II) yeast, (III) control, (IV) yeast, (V) control. The average production of 4% FCM and the average butterfat percentage for each period was (I) 53.3, 3.8, (II) 51.7, 3.8, (III) 53.8, 3.9, (IV) 54.0, 3.8, (V) 49.7, 3.9. The yeast-supplemented feed was not as readily consumed for the first few days after changing feeds.

A live yeast culture produced by another manufacturer was used to evaluate gas production in an artificial rumen apparatus. Alfalfa hay, alfalfa and grain, and purified cellulose (Solka-Floc) were added to strained rumen fluid. Gas production was measured on each of these substances alone and with the addition of yeast culture at the rate of 2% of the feed substrates. The yeast culture increased gas production from the purified cellulose, but had no consistent effect upon the other substrates.

Penicillin Levels in Milk from Lactating Cows Fed Various Amounts of Procaine Penicillin. S. R. SKAGGS AND D. D. MILLER, New Mexico State University, University Park.

The United States Food and Drug Administration had warned farmers that continued contamination of milk with antibiotics would bring disciplinary action by the U.S.F.D.A. Since penicillin salt had been advertised as a safeguard against bloat in cattle, it appeared that a study should be made as to the possibility that cows that received penicillin orally transmitted significant amounts to their milk.

Two lactating dairy cows were fed daily doses of procaine penicillin in salt sprinkled over their concentrate feed. Milk samples from each cow were analyzed for penicillin content by the Bacto-Concentration Disk method. No penicillin was found in the milk when the cows received 88.9 mg. (86,700 I.U.) penicillin daily. When the feeding level was increased to 177.8 mg. daily, approximately 0.05 I.U. penicillin per milliliter was found in the milk. A feeding level of 277.8 mg. daily increased the milk level to between 0.10 and 0.15 I.U. per ml. Blood samples from these cows showed approximately the same level of penicillin content as the milk samples.

A Method for Getting More Value from DHIA Records. C. J. HOFFMAN, Colorado State University, Fort Collins.

A booklet has been prepared which dairymen can use to chart and graph data from their Dairy Herd Improvement Association (DHIA) records. These charts and graphs help to determine weaknesses in herd management.

The charts help dairymen to observe the monthly production of a herd, calving interval, percentage of dry cows, number of cows removed from the herd and the reasons why, age of heifers at freshening, average age of cows in the herd, distribution of production by cows, and the monthly feed-cost chart.

Workshop sessions have been held with 16 DHIA. All have been highly successful and it has been suggested by dairymen that this be an annual program. A number of other states have learned about Colorado's approach and are in the process of developing similar programs.

Effect of Ration Fiber upon Respiration Rate and Rectal Temperature. MELCHIOR CADENA, C. AND J. V. BATEMAN, Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica.

Four daughters each, of three different milking Criollo bulls, were submitted to 35° C. and 25-mm. vapor pressure in a climatic chamber while on diets containing 16, 23, and 25.5% fiber. The effect of these fiber levels on respiration rates and rectal temperatures was studied. The correlations between hematocrit and respiration rates, and hematocrit and rectal temperatures, were determined. They were −.51 and −.74, respectively. The effect of fiber level on respiration rate was not significant, but the difference in response of different animals was highly significant. Fiber level had a significant effect upon rectal temperature. The responses of individuals were also highly significant. These results led to the following conclusions: 1) The response to heat stress with different levels of ration fiber varies with individuals. 2) Rectal temperatures change with levels of fiber; therefore, experiments relating to physiology and thermal stress should take this into account. 3) The fiber content of the ration should be reported.

Controlling Oxidized Flavor with Heat-Treated Milk. D. D. MILLER AND S. R. SKAGGS, New Mexico State University, University Park.

Theoretically, the sulfhydryl groups produced by heating milk should act as reducing agents and, thereby, inhibit the production of oxidized flavor. This work was done to determine the validity of the above theory.

Milk, which produced oxidized flavor spontaneously, had various amounts of the same milk heated to 90° C. for 45 min. added to the raw milk. The samples were pasteurized, cooled, and stored for seven days at 4° C. Daily organoleptic tests were made.

Inhibition of oxidized flavor was noted at the 2% level. Cooked flavor developed at the 3.5% level. Greatest inhibition, before cooked flavor developed, was observed at the 3% level. Complete prevention of oxidized flavor occurred in some samples at the 3% level, but this was dependent upon the susceptibility of the milk to oxidized flavor. When 0.1 p.p.m. copper was added to the samples, complete prevention never occurred, but good inhibition was noted at the 3% level. Heating the milk for shorter or longer periods was not as effective as 45 min. at 90° C.

Influence of Brand of Cocoa on Consumer Acceptance of Chocolate Drink. H. S. WILLARD, W. R. THOMAS, AND C. E. BERNBECK, University of Wyoming, Laramie.

To determine if brand of cocoa alters consumer acceptance of chocolate drink, between 20 and 50 college students of both sexes were given three samples successively of chocolate drink per session and were asked to express degree of likes and dislikes on a Hedonic scale. Each drink was identical except for the brand of cocoa. Fifteen series of three samples were submitted, with one brand of cocoa common to each series being used as control. Differences from the mean score of the control sample showed that consumer preference was not influenced by the per cent of fat or method of manufacture of the cocoa. Control samples appearing in different series, as a rule received somewhat the same score. On an average, processed cocoas out-scored the natural cocoas, whereas the high-fat cocoas out-scored the low-fat cocoas; but differences were of no statistical significance.

The Consumer Acceptance of Milk Beverages as Affected by Fat and Solids-not-fat Content. J. W. STULL AND J. S. HILLMAN, University of Arizona, Tucson.

A consumer-preference study was conducted to provide statistically reliable data on which standards and practices relating to the optimum composition of milk beverages can be based.

The addition of solids-not-fat (SNF) to milk beverages is not provided for in most standards. There is a general awareness, however, that the addition of from 0.5 to 2.0% SNF to either whole, low-fat, or nonfat milk beverages improves palatability, flavor, food value, and utilization.

Taste discrimination or threshold tests involving 2,132 paired and triangulation decisions were conducted with adult men and women who were not trained dairy products judges. The results showed that people could consistently differentiate between milk beverages with variations in fat and SNF contents of 0.5 and 1.0%, respectively.

Paired preference comparisons within and between each beverage class (whole, low-fat, and nonfat beverages) were made with approximately 10,000 persons in retail food markets, public gatherings, and public schools. Beverages with 0.5 to 1.0% added SNF were preferred. There was no significant difference in preference for a regular whole milk (3.5% fat, 8.5% SNF) when compared with a low-fat beverage (2.0% fat, 10% SNF).

BOOK REVIEWS

METABOLISM IN THE RUMEN. E. F. Annison and Dyfed Lewis. John Wiley & Sons, Inc., N. Y. 184 pp. 1959.

This small volume, the latest addition to the series of Meuthuen's monographs on biochemical subjects, will be welcomed by ruminant nutritionists as the first published work devoted entirely to rumen metabolism. It should also be of interest to biochemists and microbiologists in related areas, because of the unique biological organization of the rumen.

It is regrettable in one sense that a more comprehensive volume was not undertaken, in which rumen physiology and the broad field of ruminant nutrition also were encompassed. However, in confining their coverage to metabolic activities of the rumen, the authors have achieved their objectives extremely well, and the book will provide a valuable reference aid both in teaching and in research.

The book is organized into six chapters dealing with various aspects of metabolism in the rumen. Chapter 2, on the microbiology of the rumen, provides an excellent summary of the types and characteristics of microorganisms found in the rumen, together with a discussion of the biological parameters of the rumen that exert control on the microbial population. This chapter should be especially valuable to the many students and investigators of ruminant nutrition whose primary interests are in areas other than bacteriology. The greater part of the text deals with the metabolism of carbohydrates and nitrogen in the rumen. Unquestionably, this is where the emphasis belongs. An entire chapter is devoted to absorption from the rumen, with subdivisions dealing with the absorption of volatile fatty acids, ammonia, glucose and lactic acid, gases, and inorganic ions and water. A discussion of techniques used in absorption studies and difficulties in measuring absorption rates also is included. The concluding chapter deals with ruminant disorders associated with metabolic disturbances in the rumen and, although none of the disorders are discussed comprehensively, sufficient data are presented to adequately link the particular disorder to some rumen metabolic activity.

This book will make a valuable supplemental text for courses in animal nutrition and, while it is limited in scope, the price is modest.

R. E. BROWN
University of Illinois
Urbana

ELEMENTS OF DAIRYING LABORATORY MANUAL. E. E. Bartley, F. E. Eldridge, E. L. Farmer, F. C. Fontaine, and G. B. Marion. Burgess Publishing Company, Minneapolis, Minnesota. 2nd ed. 1959.

This laboratory manual was prepared for use in the college introductory course in dairying. In it, 11 exercises of the type that would permit maximum student participation are outlined.

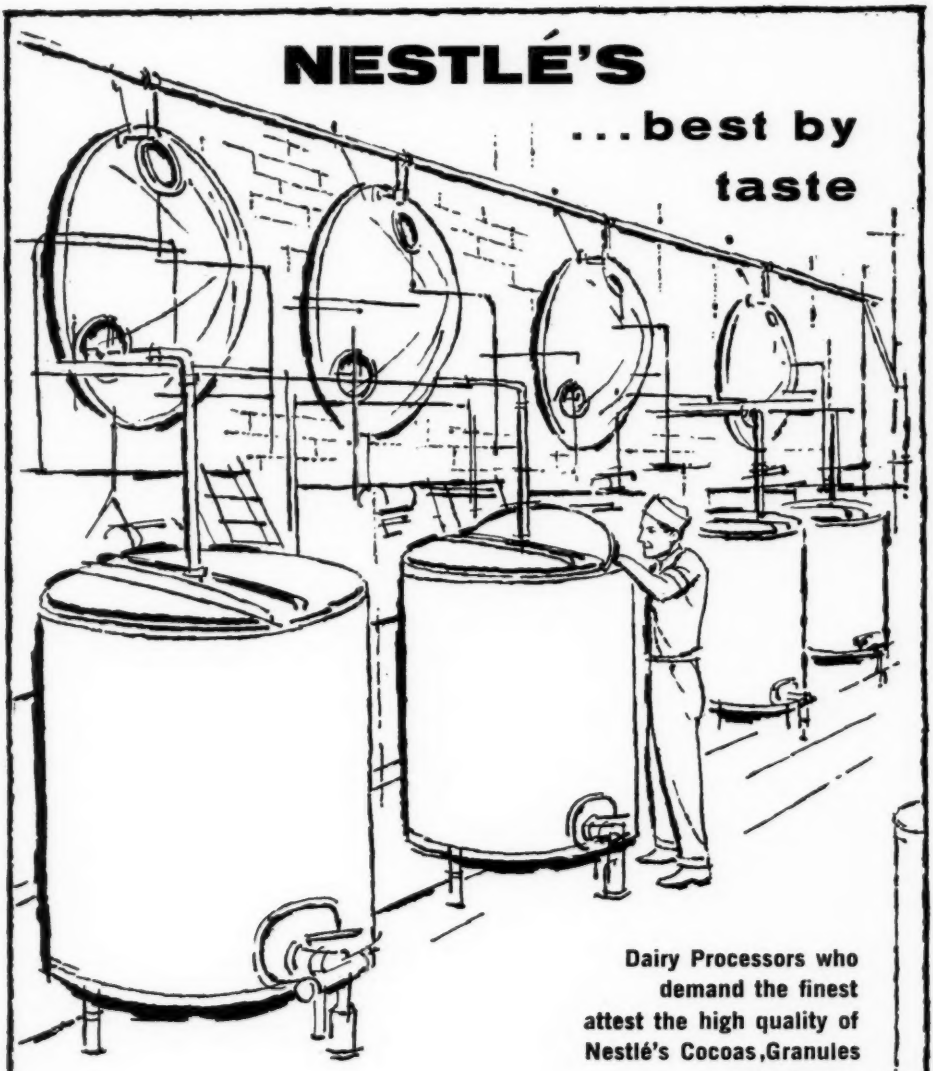
The subject matter areas covered and the number of exercises devoted to each include: Composition of Milk, 2; Tour of the Dairy Processing Plant, 1; Quality of Dairy Products, 1; Feeding Dairy Cows, 3; Management and Housing, 1; Milking Management, 1; Artificial Breeding, 1; and Dairy Cattle Judging, 1.

The exercises are well illustrated with pictures and schematic drawings, along with procedures and problems.

K. A. KENDALL
University of Illinois
Urbana

NESTLÉ'S

...best by taste



Dairy Processors who demand the finest attest the high quality of Nestlé's Cocoas, Granules and Liquors.

PETER'S® • RUNKEL'S®

NESTLÉ'S®

THE NESTLÉ COMPANY, INC.
WHITE PLAINS, N. Y.

®Trade Mark Reg.

DIFCO

Antisera and Antigens

Enteropathogenic Coli Klebsiella

Salmonella Shigella

Brucella Leptospira Streptococci

*

C Protein Antiserum and Standard

*

Infectious Mononucleosis Antigens and Standards

Potent

Stable

Specific

Descriptive literature available on request

Specify

DIFCO LABORATORY PRODUCTS

BIOLOGICS CULTURE MEDIA REAGENTS